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EDITORIAL

Strategy for improving survival and reducing recurrence of HCV-related hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer-related death in the world. With advances in imaging diagnostics, accompanied by better understanding of high-risk patients, HCC is now frequently detected at an early stage; however, the prognosis remains poor. The recurrence rate after treatment of HCC is higher than that associated with cancers of other organs. This may be because of the high incidence of intrahepatic distant recurrence and multicentric recurrence, especially with hepatitis C virus (HCV)-related hepatocellular carcinoma. The Barcelona Clinic Liver Cancer (BCLC) classification has recently emerged as the standard classification system for the clinical management of patients with HCC. According to the BCLC staging system, curative therapies (resection, transplantation, transcatheter arterial chemoembolization, percutaneous ethanol injection therapy, percutaneous microwave coagulation therapy and percutaneous radiofrequency ablation) can improve survival in HCC patients diagnosed at an early stage and offer a potential long-term cure. However, treatment strategies for recurrent disease are not mentioned in the BCLC classsification. The strategy for recurrence may differ according to the recurrence pattern, *i.e.*, intrahepatic distant recurrence *vs* multicentric recurrence. In this article, we review recurrent HCC and the therapeutic strategies for reducing recurrent HCC, especially HCV-related HCC.

Key words: Hepatocellular carcinoma; Intrahepatic distant recurrence; Multicentric recurrence; Hepatitis C virus; Interferon; Arterial chemotherapy

Core tip: Recent advances in treatment modalities have improved the survival rate of patients with hepatocellular carcinoma (HCC). However, long-term outcomes of patients with HCC remain unsatisfactory because of the high incidence of distant intrahepatic recurrence, multicentric recurrence and low survival rates. In particular, hepatitis C virus-related hepatocellular carcinoma has a much higher recurrence rate than other cancers. In this article, we describe the prognosis of recurrent HCC and the therapeutic strategies for reducing recurrent HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer mortality in the world[1,2]. It is estimated that HCC is responsible for more than 600000 deaths annually worldwide[3].

Recent advances in treatment modalities have improved the survival rate of patients with HCC[4,5]. However, long-term outcomes for patients with HCC remain unsatisfactory because of the high incidence of intrahepatic distant recurrence, multicentric recurrence and low

survival rates.

In many cases, surgical options for HCC are limited because of complicating hepatic cirrhosis; furthermore, HCC is associated with a 5-year recurrence rate of approximately 80% after radical treatment, which is much higher than that of other gastrointestinal carcinomas, resulting from its tendency to multicentric carcinogenesis secondary to chronic liver disease, or intrahepatic distant recurrence[6,7].

Typically, recurrence rates in HCC follow a 2-peak distribution. Early recurrence usually occurs within 2 years after resection, and is most closely related to cancer metastasis, while late recurrence mainly results from *de novo* tumors as a consequence of the carcinogenic cirrhotic environment[7]. Therefore, a treatment strategy with a focus on recurrence is necessary.

In recent years, the Barcelona Clinic Liver Cancer (BCLC) classification has emerged as the standard classification system for the clinical management of patients with HCC[8]. However, in the recommendations regarding topical therapy for the treatment of early stage HCC, the BCLC guidelines do not mention a strategy for reducing recurrence. Togo *et al*[9] recommended a strategy based on the differentiation between recurrence types, *i.e.*, intrahepatic distant recurrences *vs* multicentric recurrence. Transcatheter arterial infusion (TAI) with platinum agents may be effective as adjuvant therapy for the prevention of residual liver recurrence after hepatectomy, probably by suppression of the development of intrahepatic micrometastasis, rather than multicentric carcinogenesis. Furthermore, antiviral treatment, including interferon (IFN), is recommended for preventing multicentric recurrence. We discuss a possible strategy for reducing the recurrence of hepatitis C virus (HCV)-related HCC in terms of our clinical data.

PREVENTIVE TREATMENT STRATEGY PRIOR TO DEVELOPMENT OF RECURRENT MULTIPLE HEPATOCELLULAR CARCINOMA TO MAINTAIN RESIDUAL HEPATIC FUNCTION

IFN therapy may be useful in the prevention of recurrence of HCC secondary to chronic viral hepatitis after radical treatment by inhibiting multicentric carcinogenesis, while chemotherapy based on TAI is recommended to inhibit intrahepatic metastasis.

It is widely recognized that HCV infection is a major cause of liver cirrhosis and HCC in Japan and other countries. According to the Liver Cancer Study Group of Japan, 67.7% of Japanese patients with HCC are HCV antibody-positive[10].

We evaluated the treatment response and functional hepatic reserve in patients who received combination therapy with PEG-IFN α-2b and ribavirin (RBV) after radical treatment of HCV-related HCC[11].

This study comprised 54 patients with primary HCV-related HCC (stage Ⅰ/Ⅱ) whose survival rate, metachronous recurrence rate and hepatic functional reserve were assessed. Among these patients, 29 received combination therapy with PEG-IFN α-2b and RBV after treatment of HCC (secondary IFN treatment group), and the other 25 did not receive IFN, including PEG-IFN α-2b and RBV (non-secondary treatment group). The 1- and 3-year cumulative survival rates were 100.0% and 90.2% in the secondary IFN treatment group, and 96.0% and 61.2% in the non-secondary treatment group, respectively, showing a significant difference between the groups. Multivariate analysis identified secondary IFN treatment as a significant factor related to prognosis. In the PEG-IFN α-2b/RBV group, serum albumin levels decreased transiently but increased thereafter, indicating improvement in hepatic functional reserve. These results show that combination therapy with IFN, including PEG-IFN α-2b and RBV, following treatment of HCC, contributes to improvement in hepatic functional reserve and increases treatment options in the case of recurrence.

TREATMENT STRATEGIES FOR INTRAHEPATIC METASTASIS

For intrahepatic metastasis, on the other hand, it is essential to select treatment not only for overt lesions, but also for micrometastasis. Hence, combination therapy, including intra-arterial treatment, such as transcatheter arterial chemoembolization (TACE), is required for initial treatment or treatment of recurrence.

Efficacy of platinum-containing drugs in transhepatic arterial infusion

After treatment of HCC, the remaining liver is still in a state of precarcinogenesis. The protective effect of chemotherapy against recurrence in the remaining liver is demonstrated by its efficacy in patients with a high probability of intrahepatic metastasis, and thus, a high recurrence rate.

Although it is well-established that more effective chemotherapy, performed preoperatively in patients with intrahepatic micrometastasis or with the possibility of intraoperative tumor spread, may prevent tumor recurrence in the residual liver and further improve prognosis, few reports have described such cases. Systemic chemotherapy is generally not effective in most cases of HCC. Further, chemotherapy often impairs liver function in cases complicated by cirrhosis. At present, systemic chemotherapy with cytotoxic anticancer agents is only used infrequently in the treatment of HCC. Compared with systemic chemotherapy, hepatic arterial infusion (HAI) chemotherapy has the advantages of increasing the local concentration of chemotherapeutic agents to levels that are adequate to kill cancer cells without damaging healthy

liver tissue, and of reducing systemic side effects.

Treatment strategy and problems with TACE

In Japan, TACE, which is recommended for inoperable patients in whom local puncture therapy is not indicated according to the guidelines for the management of liver cancer, plays a central role in the treatment of recurrent advanced multiple HCC[12,13].

TACE is primarily performed as chemolipiodolization, using anticancer drugs mixed with lipiodol[14,15]. There have also been several retrospective reports of TACE with anthracyclines *vs* platinum compounds, suggesting the utility of platinum compounds[16,17]. However, no single effective drug has been identified, because no prospective comparative studies have been performed, and various anticancer drugs, including epirubicin, cisplatin, mitomycin C and doxorubicin, have been used with intercenter variance.

Kaibori *et al*[18] previously recommended that preoperative whole-liver chemolipiodolization reduces postoperative recurrence and prolongs survival in patients undergoing resection of hepatocellular carcinoma.

However, they subsequently performed a randomized controlled trial to evaluate the influence of preoperative TACE on survival after the resection of HCC, following which they concluded that preoperative selective TACE and whole-liver chemolipiodolization plus TACE do not reduce the incidence of postoperative recurrence or prolong survival in patients with resectable HCC[19].

While intra-arterial chemotherapy is not highly appreciated in the West, HAI resulting in high local drug concentrations, is expected to improve the prognosis and prevent disease recurrence, because lesions are often localized to the liver even in advanced stages of HCC. However, various therapeutic regimens have been tried, without reaching a consensus regarding the administration method or dose level.

We previously reported that platinum agents, such as cisplatin, which are widely used for the treatment of a variety of malignancies, may be effective for HCC treatment.

In Japan, a fine powder formulation of cisplatin (cisplatin powder) (DDPH, IA call; Nippon Kayaku, Tokyo, Japan) was developed in 2004 and approved for the treatment of HCC *via* a transarterial approach, without lipiodol or embolic material. Cisplatin powder is readily soluble and more suitable for the preparation of high-concentration (about three times) aqueous solutions (1.4 mg/mL) than conventional cisplatin formulations (0.5 mg/mL). Therefore, a single session of TAI therapy with cisplatin powder has the benefit of increasing drug concentration locally in the HCC, and is expected to have a high therapeutic efficacy.

We evaluated the effectiveness of additional chemotherapy with the platinum-containing drugs carboplatin (CBDCA) and DDPH in preventing intrahepatic distant tumor recurrence[20].

Seventy-eight patients with a diagnosis of primary stage Ⅰ / Ⅱ HCC who underwent TACE and RFA after whole liver arterial infusion of CBDCA (25 patients) or DDPH (53 patients) for local control and recurrence prevention were followed up on a long-term basis. The clinical background factors, intrahepatic distant tumor recurrence rate, and intrahepatic distant tumor recurrence factors were compared between the CBDCA and DDPH groups. While no significant differences in background clinical characteristics were observed between the two groups, the intrahepatic distant tumor recurrence rate was significantly lower in the DDPH group[20]. In multivariate analysis using Cox's proportional hazard model, whole liver arterial infusion of DDPH was identified as an independent factor for the prevention of recurrence, *i.e.*, whole liver arterial infusion of DDPH significantly prevented intrahepatic distant tumor recurrence. Significant prevention of recurrence by a single infusion of DDPH compared with CBDCA suggests the utility of DDPH-based treatment strategies in patients with intrahepatic metastasis.

However, no evidence of its contribution to patient survival has been found, and TAI is not mentioned in any Western guidelines[21]. Nevertheless, it has been shown that some patients with TACE-refractory HCC were responsive to repeated TAI, with survival being prolonged in these responsive patients. According to the 18th nationwide follow-up survey of primary liver cancer, 85.8% of 1862 Japanese HCC patients treated with chemotherapy underwent TAI[10]. TAI is relatively often selected as the final choice for advanced HCC, including recurrent HCC; therefore, it is important to improve the response rate to TAI.

Thus, treatment strategies with DDPH-based TAI need to be established by conducting prospective randomized control trials[22,23].

CONCLUSION

Currently, the efficacy of tertiary prevention of HCC with any agent, including chemotherapy, HCV therapy, or IFN, has yet to be proven, and safe and effective chemotherapy for HCC-recurrence has yet to be established.

In this article, we reviewed our strategy for improving survival and reducing the recurrence of HCV-related HCC.

When deciding on treatment strategies for recurrence of HCV-related HCC, it is very important to select appropriate treatment according to the degree of disease progression, and to determine the patient's functional hepatic reserve. The type of recurrence and previous treatments also should be taken into consideration. It is also imperative to establish preventive strategies against precarcinogenesis associated with multicentric carcinogenesis and residual intrahepatic metastasis as early as possible, thereby improving prognosis.

REFERENCES

1 **Shariff MI**, Cox IJ, Gomaa AI, Khan SA, Gedroyc W, Taylor-Robinson SD. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and

therapeutics. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 353-367 [PMID: 19673623 DOI: 10.1586/egh.09.35]
2 **Mazzaferro V**, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543-1554 [PMID: 17133492 DOI: 10.1002/hep.21415]
3 **Ferenci P**, Fried M, Labrecque D, Bruix J, Sherman M, Omata M, Heathcote J, Piratsivuth T, Kew M, Otegbayo JA, Zheng SS, Sarin S, Hamid SS, Modawi SB, Fleig W, Fedail S, Thomson A, Khan A, Malfertheiner P, Lau G, Carillo FJ, Krabshuis J, Le Mair A. Hepatocellular carcinoma (HCC): a global perspective. *J Clin Gastroenterol* 2010; **44**: 239-245 [PMID: 20216082 DOI: 10.1097/MCG.0b013e3181d46ef2]
4 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
5 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]
6 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-77; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]
7 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409 DOI: 10.1016/S0168-8278(02)00360-4]
8 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]
9 **Togo S**, Tanaka K, Masui H, Matsuo K, Morioka D, Kurosawa H, Miura Y, Endo I, Sekido H, Shimada H. Usefulness of prophylactic transcatheter arterial infusion of anticancer agents with lipiodol to prevent recurrence of hepatocellular carcinoma after hepatic resection. *Int Surg* 2005; **90**: 103-108 [PMID: 16119716]
10 **Ikai I**, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M, Kudo M. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007; **37**: 676-691 [PMID: 17617112]
11 **Ishikawa T**, Higuchi K, Kubota T, Seki K, Honma T, Yoshida T, Kamimura T. Combination PEG-IFN a-2b/ribavirin therapy following treatment of hepatitis C virus-associated hepatocellular carcinoma is capable of improving hepatic functional reserve and survival. *Hepatogastroenterology* 2012; **59**: 529-532 [PMID: 22024226 DOI: 10.5754/hge10867]
12 **Kokudo N**, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: the J-HCC guidelines. *J Gastroenterol* 2009; **44** Suppl 19: 119-121 [PMID: 19148805 DOI: 10.1007/s00535-008-2244-z]
13 **Kudo M**, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, Kojiro M, Makuuchi M. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011; **29**: 339-364 [PMID: 21829027 DOI: 10.1159/000327577]
14 **Satake M**, Uchida H, Arai Y, Anai H, Sakaguchi H, Nagata T, Yamane T, Kichikawa K, Osaki Y, Okazaki M, Higashihara H, Nakamura H, Osuga K, Nakao N, Hirota S. Transcatheter arterial chemoembolization (TACE) with lipiodol to treat hepatocellular carcinoma: survey results from the TACE study group of Japan. *Cardiovasc Intervent Radiol* 2008; **31**: 756-761 [PMID: 18389187 DOI: 10.1007/s00270-007-9255-7]
15 **Takayasu K**, Arii S, Ikai I, Kudo M, Matsuyama Y, Kojiro M, Makuuchi M. Overall survival after transarterial lipiodol infusion chemotherapy with or without embolization for unresectable hepatocellular carcinoma: propensity score analysis. *AJR Am J Roentgenol* 2010; **194**: 830-837 [PMID: 20173167 DOI: 10.2214/AJR.09.3308]
16 **Yodono H**, Matsuo K, Shinohara A. A retrospective comparative study of epirubicin-lipiodol emulsion and cisplatin-lipiodol suspension for use with transcatheter arterial chemoembolization for treatment of hepatocellular carcinoma. *Anticancer Drugs* 2011; **22**: 277-282 [PMID: 21360850 DOI: 10.1097/CAD.0b013e328342231d]
17 **Kasai K**, Ushio A, Sawara K, Miyamoto Y, Kasai Y, Oikawa K, Kuroda H, Takikawa Y, Suzuki K. Transcatheter arterial chemoembolization with a fine-powder formulation of cisplatin for hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 3437-3444 [PMID: 20632449]
18 **Kaibori M**, Tanigawa N, Matsui Y, Kwon AH, Sawada S, Kamiyama Y. Preoperative chemolipiodolization of the whole liver for hepatocellular carcinoma. *Anticancer Res* 2004; **24**: 1929-1933 [PMID: 15274379]
19 **Kaibori M**, Tanigawa N, Kariya S, Ikeda H, Nakahashi Y, Hirohara J, Koreeda C, Seki T, Sawada S, Okazaki K, Kwon AH. A prospective randomized controlled trial of preoperative whole-liver chemolipiodolization for hepatocellular carcinoma. *Dig Dis Sci* 2012; **57**: 1404-1412 [PMID: 22271410 DOI: 10.1007/s10620-012-2029-3]
20 **Ishikawa T**, Higuchi K, Kubota T, Seki K, Honma T, Yoshida T, Kamimura T. Prevention of intrahepatic distant recurrence by transcatheter arterial infusion chemotherapy with platinum agents for stage I/II hepatocellular carcinoma. *Cancer* 2011; **117**: 4018-4025 [PMID: 21365625 DOI: 10.1002/cncr.25989]
21 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
22 **Ishikawa T**, Imai M, Kamimura H, Tsuchiya A, Togashi T, Watanabe K, Seki K, Ohta H, Yoshida T, Kamimura T. Improved survival for hepatocellular carcinoma with portal vein tumor thrombosis treated by intra-arterial chemotherapy combining etoposide, carboplatin, epirubicin and pharmacokinetic modulating chemotherapy by 5-FU and enteric-coated tegafur/uracil: a pilot study. *World J Gastroenterol* 2007; **13**: 5465-5470 [PMID: 17907289]
23 **Ishikawa T**. Future perspectives on the treatment of hepatocellular carcinoma with cisplatin. *World J Hepatol* 2009; **1**: 8-16 [PMID: 21160960 DOI: 10.4254/wjh.v1.i1.8]

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REVIEW

Transjugular intrahepatic portosystemic shunt for the management of acute variceal hemorrhage

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Abstract

Acute variceal hemorrhage, a life-threatening condition that requires a multidisciplinary approach for effective therapy, is defined as visible bleeding from an esophageal or gastric varix at the time of endoscopy, the presence of large esophageal varices with recent stigmata of bleeding, or fresh blood visible in the stomach with no other source of bleeding identified. Transfusion of blood products, pharmacological treatments and early endoscopic therapy are often effective; however, if primary hemostasis cannot be obtained or if uncontrollable early rebleeding occurs, transjugular intrahepatic portosystemic shunt (TIPS) is recommended as rescue treatment. The TIPS represents a major advance in the treatment of complications of portal hypertension. Acute variceal hemorrhage that is poorly controlled with endoscopic therapy is generally well controlled with TIPS, which has a 90% to 100% success rate. However, TIPS is associated with a mortality of 30% to 50% in such a setting. Emergency TIPS should be considered early in patients with refractory variceal bleeding once medical treatment and endoscopic sclerotherapy failure, before the clinical condition worsens. Furthermore, admission to specialized centers is mandatory in such a setting and regional protocols are essential to be organized effectively. This review article discusses initial management and then focuses on the specific role of TIPS as a primary therapy to control acute variceal hemorrhage, particularly as a rescue therapy following failure of endoscopic approaches.

Key words: Cirrhosis; Portal hypertension; Transjugular intrahepatic portosystemic shunt; Variceal hemorrhage

Core tip: The transjugular intrahepatic portosystemic shunts (TIPS) is a highly effective treatment for bleeding esophageal and gastric varices with control of the bleeding in over 90% of the patients. Many papers have been published in the last decade that led to technical improvements and definition of the best indications for this promising treatment of complications of portal hypertension. The purpose of this article is to describe the different treatment options for patients with refractory esophageal and gastric varices bleeding and the role of TIPS as a rescue therapy. Technical aspects of this procedure and the current indications are also discussed.

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INTRODUCTION

Acute variceal hemorrhage is a common clinical emergency and most often is caused by cirrhosis-related portal hypertension[1]. Less common causes include splenic vein thrombosis, hepatic veno-occlusive disease, and primary biliary cirrhosis[1]. It is defined as visible bleeding from an esophageal or gastric varix at the time of endoscopy, the presence of large esophageal varices with recent stigmata of bleeding or fresh blood visible in the stomach with no other source of bleeding identified[1]. The frequency of gastroesophageal varices in cirrhosis varies from 30% to 70% with bleeding occurring in approximately one-third of patients[2]. Twenty percent of cirrhotics with acute variceal hemorrhage die within 6 wk[3]. The rebleeding rates range from 30% to 40% at 6 wk and the mortality from rebleeding reaches 30%[4]. Gastroesophageal varices account for approximately 80% of all cases of variceal hemorrhage[2,5]. The precipitating cause for hemorrhage, presumably an acute rise in portal pressure and subsequent variceal rupture, remains uncertain. However, several factors have been implicated including raised intra-abdominal pressure, bacterial infection, continued excess alcohol consumption and postprandial increase in splanchnic blood flow[4,5]. Predictive factors for variceal hemorrhage include a hepatic venous pressure gradient (HVPG) of > 20 mmHg[6,7], the presence of large varices with red signs[8] and underlying severe liver disease (Child-Pugh grade C)[2].

Optimal management of variceal hemorrhage requires a multidisciplinary approach involving a team of gastroenterologists, hepatologists, critical care physicians, surgeons, and interventional radiologists. The principal components of therapy include airway maintenance, hemodynamic stabilization, control of the variceal hemorrhage, and alteration of the hemodynamic effects of portal hypertension. Treatment options for the management of acute variceal hemorrhage include endoscopic therapy, use of vasoactive drugs, balloon tamponade and esophageal transaction. These various methods, either alone or in combination, are effective in controlling acute variceal hemorrhage in 80% to 90% of patients[3]. Patients who do not respond to these measures are referred for rescue therapies, which include transjugular intrahepatic portosystemic shunt (TIPS) and surgical portosystemic shunts with or without splenectomy. Because of the higher mortality of surgery in the acute setting, TIPS is the favored rescue procedure for uncontrolled variceal hemorrhage[6].

The purpose of this review is to describe the different therapeutic options available to control acute variceal hemorrhage and then to focus on the potential role of TIPS as a primary therapy to control acute variceal hemorrhage, particularly as a rescue therapy following failure of endoscopic approaches.

INDICATIONS-GASTROINTESTINAL BLEEDING

TIPS has been used to treat many complications related to portal hypertension. The relative efficacy of TIPS has been tested with randomized controlled trials (variceal bleeding, refractory ascites), whereas other indications have been evaluated in uncontrolled case series.

The causes of gastrointestinal hemorrhage in a patient with portal hypertension may be variceal rupture, portal hypertension gastropathy, postsclerotherapy ulcers, peptic ulcer disease, hemorrhagic gastritis, and Mallory-Weiss tear. TIPS is generally accepted as a second-line therapy after failure of endoscopic and medical therapy of bleeding from gastroesophageal varices[9].

Primary prophylaxis of variceal bleeding

Bleeding from esophageal varices is a common and severe complication of portal hypertension. Prevention of the initial bleeding can be achieved in a number of cases by endoscopic variceal ligation or β-blocker treatment. However, TIPS has never been tested in this situation as the use of surgical portacaval shunts has demonstrated that this approach is associated with higher morbidity and mortality rates[10].

Bleeding from gastric varices is often severe and difficult to control, particularly when fundal varices are involved. The first-line treatment is endoscopic sclerotherapy with cyanoacrylate[11]. TIPS has been used in a number of uncontrolled trials in patients in whom endoscopic therapy failed[12,13]. A recent controlled trial has shown that TIPS is more efficient than cyanoacrylate in prevention of rebleeding (secondary prophylaxis) from large gastric varices[14]. This finding, although interesting, must be confirmed by after a long-term follow-up. Importantly, due to the large size of fundal varices, the risk of rupture is still present even at a low portacaval gradient (< 12 mmHg) after TIPS[15]. This is probably best explained by the relationship between the variceal tension (and therefore the risk of rupture) and the variceal size. For this reason, it is recommended to embolize gastric varices at the time of TIPS placement[10,16].

Acute variceal bleeding

When initial bleeding occurs, it is usually controlled with less invasive endoscopic treatment and/or pharmacological therapy. In the rare instance when bleeding remains uncontrollable, TIPS has been used as a rescue treatment with good results. However, prognosis relies on the general condition of the patient, the value of the liver function reserve, and the associated comorbidities[17-20]. However, a recent randomized controlled trial evaluated the use of emergent TIPS as compared to standard medical therapy in patients with severe portal hypertension and a

Table 1 Transjugular intrahepatic portosystemic shunt *vs* endoscopic therapy in the prevention of rebleeding: Results from meta-analyses *n* (%)

Study finding	Reference, value	
	Burroughs *et al*[34]	Zheng *et al*[35]
Patients	948	883
TIPS	472	440
Endoscopic therapies	476	443
Randomized controlled trials	13	12
Recurrent bleeding		
TIPS	88 (18.6)	86 (19.0)
Endoscopic therapy	210 (44.1)	194 (43.8)
OR (95%CI) for TIPS	0.30 (0.21-0.44)	0.32 (0.24-0.43)
Post-treatment encephalopathy		
TIPS	134 (28.4)	148 (33.6)
Endoscopic therapy	83 (17.4)	86 (19.4)
OR (95%CI) for TIPS	2.08 (1.49-2.94)	2.21 (1.61-3.03)
All-cause mortality		
TIPS	130 (27.5)	111 (25.2)
Endoscopic therapy	118 (24.8)	98 (22.1)
OR (95%CI) for TIPS	1.14 (0.85-1.54)	1.17 (0.85-1.61)

TIPS: Transjugular intrahepatic portosystemic shunt.

Child-Pugh score of 7-13[21]. Treatment failure was more frequent in the medical group (50% *vs* 12%) and the survival rate was better in the TIPS group (11% *vs* 38%)[21]. This approach could justify the use of TIPS early after bleeding in patients with moderate or severe liver failure and severe portal hypertension. Current evidence supports the use of TIPS not as a primary form of treatment, but rather as a rescue treatment for patients with bleeding esophageal varices who failed pharmacological and endoscopic treatments.

Secondary prophylaxis of variceal bleeding

The strongest evidence in favor of performing a TIPS procedure exists for the secondary prevention of variceal bleeding. Twelve randomized controlled trials have been published on this topic, describing results for 948 patients, 472 of whom received a TIPS[22-33]. Recent meta-analyses found a more than threefold decrease in the risk of recurrent bleeding after insertion of a TIPS compared with various forms of endoscopic therapy (Table 1)[34,35]. Rates of rebleeding after insertion of TIPS ranged from 9.0% to 40.6%. Conversely, continued endoscopic therapy resulted in a 20.5% to 60.6% rate of rebleeding. All-cause mortality rates were similar between the TIPS and endoscopic therapy groups. However, there was a more than twofold increase in the rate of development of hepatic encephalopathy after a TIPS procedure[22-33].

Ectopic varices

Varices may develop anywhere along the digestive tract in patients with portal hypertension (duodenum, jejunum, colon, rectum) and may bleed. Local treatments are either impossible or associated with a high rate of rebleeding. The best approach is the TIPS procedure, which can be combined with embolization of the varices[36,37].

Table 2 Contraindications to placement of a transjugular intrahepatic portosystemic shunt

Absolute	Relative
Congestive heart failure	Portal vein thrombosis
Severe pulmonary hypertension	Hepatocellular carcinoma
Severe systemic sepsis	Severe coagulopathy
Unrelieved biliary obstruction	Hepatic encephalopathy
Severe tricuspid regurgitation	Obstruction of all hepatic veins

Portal hypertensive gastropathy

These gastric lesions rarely induce problematic bleeding. Nonetheless, anecdotal case reports have suggested that TIPS may control bleeding in these patients[38].

Gastric antral vascular ectasia

Chronic bleeding from gastric antral vascular ectasia may be difficult to manage. However, TIPS does not help to control this type of hemorrhage, probably because these vascular lesions are related to liver disease and not to portal hypertension[38,39].

Other indications

Despite limited evidence, TIPS has found wider clinical use than just secondary prevention of variceal bleeding, treatment of refractory acute variceal bleeding and management of refractory ascites. These clinical indications include Budd-Chiari syndrome[40,41], hepatic veno-occlusive[42], hepatic hydrothorax[43-46], hepatorenal syndrome[47,48], and hepatopulmonary syndrome[49].

CONTRAINDICATIONS

Absolute contraindications to TIPS include right heart failure and pulmonary arterial hypertension. The TIPS survival benefit in patients with severe liver failure (Child-Pugh class C cirrhosis, model for end-stage liver disease score > 22, serum bilirubin > 3 mg/dL) also remains unclear. Relative contraindications include hepatic encephalopathy (which may worsen following TIPS creation), polycystic liver disease (technically challenging with a high incidence of hemorrhagic complications), active sepsis (poor outcomes), and chronic organized portal vein thrombosis (technically challenging for successful TIPS creation). Acute portal vein thrombus is not a contraindication for TIPS, but it necessitates extensive stenting to prevent shunt occlusion[50]. The contraindications are summarized in Table 2.

PRE-TIPS TREATMENT OPTIONS FOR ACUTE VARICEAL BLEEDING

Initial management

As with all acutely unwell patients, the basic resuscitation pathway (airway, breathing, circulation) should be instigated. Initially, the airway and breathing should be

assessed. Endotracheal intubation should be considered early, especially in patients who are deemed at high risk for aspiration, that is, those demonstrating signs of encephalopathy or ongoing severe uncontrolled hemorrhage. The adequacy of filling of the circulation should then be assessed and two large bore intravenous cannula inserted before placement of a central line. Plasma expanders and packed red blood cells should be used to replace volume loss and any underlying coagulopathy corrected with platelets and fresh-frozen plasma. Despite portal pressure correlating directly with plasma volume, all cirrhotic patients with variceal hemorrhage should be maintained at a normal central venous pressure, while avoiding under filling the circulation in order to "keep the portal pressure low"[51]. Ideally, these patients should be admitted to an intensive care or high dependency unit where cardiac monitoring and high intensity nursing are readily available. All patients with cirrhosis and gastrointestinal bleeding are at an increased risk of bacterial infection and thus prophylactic antibiotics should be administered[52,53]. Several meta-analyses have demonstrated a reduction in bacterial infections and improved survival attributed to the use of short-term prophylactic antibiotics[54]. No consensus exists as to which antibiotic should be given but intravenous quinolones are generally recommended for 5-7 d followed by oral quinolones[55-57].

Endoscopic therapy

Sclerotherapy and variceal band ligation are the two endoscopic interventions currently used. Endoscopic sclerotherapy involves a sclerosant such as ethanolamine injected directly into the bleeding varix. Variceal band ligation is associated with fewer side effects than sclerotherapy. Banding devices that allow multiple bands to be applied without repeated reintroduction of the endoscope should be used. Variceal band ligation is the preferred endoscopic therapy for the secondary prevention of esophageal variceal hemorrhage and most centers now also use band ligation to control acute bleeding[58].

Pharmacological treatment

Various pharmacological agents, including vasopressin, somatostatin, octreotide and terlipressin, are of benefit in acute variceal bleeding[59-61]. These drugs cause splanchnic vasoconstriction and thus reduce portal flow. They are particularly useful when an out-of-hours endoscopy service is unavailable. Temporary cessation of bleeding and reduction in treatment failure has been reported with early administration of these drugs[62]. An ongoing debate does continue about the efficacy of these agents, particularly vasopressin analogues, as they are not without significant side effects such as increased risk of mesenteric ischemia and myocardial infarction. These agents should therefore be used with caution in patients with known atheromatous disease. Vasopressin is no longer used alone and rarely with nitrates, with terlipressin being the current agent of choice. Because a significant proportion of patients suspected of variceal hemorrhage will actually be bleeding from nonvariceal sources, widespread use of vasoactive drugs before endoscopy should be discouraged, as is diagnostic endoscopy attempted by someone who is unable to perform band ligation or sclerotherapy. Combination therapy of these vasoactive agents and endoscopic therapy is becoming common but a meta-analysis of several studies, although demonstrating initial improvement in hemostasis, did not reveal a reduction in mortality with combination therapy[63].

Balloon tamponade

Balloon tamponade is invaluable in cases of uncontrolled hemorrhage when an endoscopy service is unavailable or when control cannot be achieved endoscopically. Balloon tamponade, however, is not without complications that include gross esophageal ulceration and esophageal perforation. To minimize complication rates, this procedure should be performed only by experienced staff and in the majority of cases, lone inflation of the gastric balloon should be sufficient. In the rare cases that the esophageal balloon requires inflation, inflation pressure should be closely monitored and regular deflation should also be performed. Nursing protocols should be produced and should include regular checks of the gastric balloon position and regular aspiration from both the gastric and esophageal ports. Medical staff should be alerted if blood aspiration volumes are increasing at either port. Panés *et al*[64] examined the use of esophageal tamponade in 151 cases and reported that although balloon tamponade achieved hemostasis, 50% of patients experienced rebleeding on removal of the Stenstaken-Blackmore tube. It is essential therefore that balloon tamponade is considered only as a holding measure until a definitive procedure can be performed.

TIPS PROCEDURE

Timing of salvage therapy

Although the above studies illustrate the efficacy and applicability of TIPS in the setting of uncontrolled variceal bleeding, there remains a debate about the best time to perform the procedure. Although a convenient definition of uncontrolled variceal bleeding can be taken as failure of two endoscopic treatments, this does not necessarily indicate criteria for TIPS insertion. Patients with a Child-Pugh A score and whose bleeding does not appear life threatening may be managed by balloon tamponade followed by further sessions of endoscopic band ligation and generally do not require TIPS. Conversely, patients with advanced liver disease who have had a single massive bleed and unsuccessful endoscopic treatment on one occasion and require balloon tamponade, may be better treated by TIPS early rather than undergoing a second endoscopic therapy session. Monescillo *et al*[65] showed that early insertion of TIPS might confer extra benefit. The basis of this is probably due to reducing the duration

or risk of hypotension that is likely to be detrimental for patients with decompensated liver disease.

Pre-procedural imaging

Any prior imaging studies (ultrasound, computed tomography, magnetic resonance imaging), should be reviewed to confirm portal vein patency and to assess the presence of gastroesophageal varices and other porto-systemic shunts that may compete with the TIPS. The location of the portal vein bifurcation should be determined based on prior imaging, as an extrahepatic portal vein bifurcation occurs in 25% of patients and accessing an extrahepatic portal vein during TIPS carries a high mortality[10,66]. Imaging may also demonstrate the presence of splenic vein thrombosis, for which TIPS is not the treatment of choice, ascites, and general hepatic morphology. If there is large-volume ascites, pre-procedural paracentesis should be performed. If no imaging is available, Doppler ultrasound assessment of the portal vein is recommended before initiating the TIPS procedure[66]. The procedure is performed under general anesthesia and thus an emergency consultation with anesthesia is initiated as soon as TIPS is considered.

Equipment specifications

The procedure room should have the necessary equipment for continuous hemodynamic monitoring as well as for anesthesia, with access to oxygen, anesthetic gases, and suction. The angiographic equipment should allow for high-resolution fluoroscopy, digital subtraction angiography (DSA), and operator-definable protocols for performing CO_2 DSA, low-frame-rate fluoroscopy, and road map imaging. A trained radiologic technologist who is familiar with the necessary catheters, guidewires, balloons, stents, and imaging equipment should be present. Anesthesia or nursing personnel are essential for patient monitoring and assistance with hemodynamic measurements. The physician operator should be an interventional radiologist who is trained in performing TIPS procedures, as these require a high level of technical expertise and knowledge of the equipment, materials, anatomy, physiology, pathology, appropriate technique, and potential complications. The operator must be able to cope with the difficulties that are often associated with emergency TIPS[6,9,50,66].

Shunt technique

Sets: Three types of TIPS sets are commercially available. Two sets, made by Cook Medical (Bloomington, IN, United States), include the "RingTIPS set" and the "Rosch-Uchida TIPS set". The RingTIPS set has a 16-G curved Colapinto needle, while the Rosch-Uchida set has a 16-G curved blunt cannula through which a 5-Fr catheter with an inner needle is advanced to access the portal vein. After using the needle to advance the catheter, the needle is removed and the catheter is slowly withdrawn while maintaining suction in the catheter. There is also a cope version of the ring set, which uses a 20- to 21-G-long needle. Another set is made by AngioDynamics (Queensbury, NY, United States) and has a hollow 21-G needle that is passed through a hollow, curved cannula.

Steps: After entry into the internal jugular vein, a catheter is introduced and guided through the superior vena cava, right atrium, and inferior vena cava into a hepatic vein. The use of the proximal portion of the hepatic vein has two purposes. The first is to utilize, for shunt creation, the largest diameter of the hepatic vein to potentially prevent or delay any outflow shunt stenosis. The second is to be sure that one begins cephalad to the desired portal vein entry site. A needle inserted through the catheter is then used to puncture the liver from a central portion of the hepatic vein and enter the main portal branch, usually the right portal vein. In the right hepatic vein, the cannula is rotated approximately 90° anteriorly and then advanced and maintained with continual caudal pressure, so that it is wedged against the wall of the hepatic vein. When in the middle hepatic vein, the cannula is rotated posteriorly in the some way. Carbon dioxide wedged hepatic venography is used to identify the portal vein[67]. Iodinated contrast medium can also be used. The puncture can be also navigated with ultrasonography. Depending on the anatomy, it might by possible to use a tract from the right hepatic vein to the left portal branch, and vice versa. The needle tract is then dilated by a balloon catheter, establishing a connection between the portal and systemic circulation directly inside the liver parenchyma. The parenchymal tract is kept open by insertion of an expandable metallic stent. A dedicated TIPS stent graft was designed to extend the covered portion to the orifice of the hepatic vein at the inferior vena cava[41]. The only uncovered part of the stent graft, which is 2 cm long, is the section that protrudes into the portal vein. This both anchors the device and allows blood to flow through the interstices of the uncovered portion to the peripheral (parenchymal) portal vein branches. The alternative to the dedicated stent graft has been a self-expandable stent used for bridging portal and hepatic veins in a similar way. The bare stents are used for patients at high risk of hepatic encephalopathy or for recanalization of the portal vein. The shunt diameter is finalized by balloon dilatation of the deployed stent graft or stent. Depending on the diameter of the expandable stent or stent graft used for TIPS creation, various amounts of portal blood are diverted into the systemic circulation, resulting in the decompression of portal hypertension. The size of the balloon catheter is usually 8 mm. Depending on the pressure gradient measured between the portal vein and right atrium after stent or stent graft placement, a larger angioplasty balloon catheter is an option to achieve adequate stepwise decompression. For liver transplant candidates, precise positioning of both ends of the stent or stent graft is critical[6,50]. The needle may exit the liver and lacerate the liver capsule or enter the hepatic artery. Embolization of the parenchymal tract is the first-line

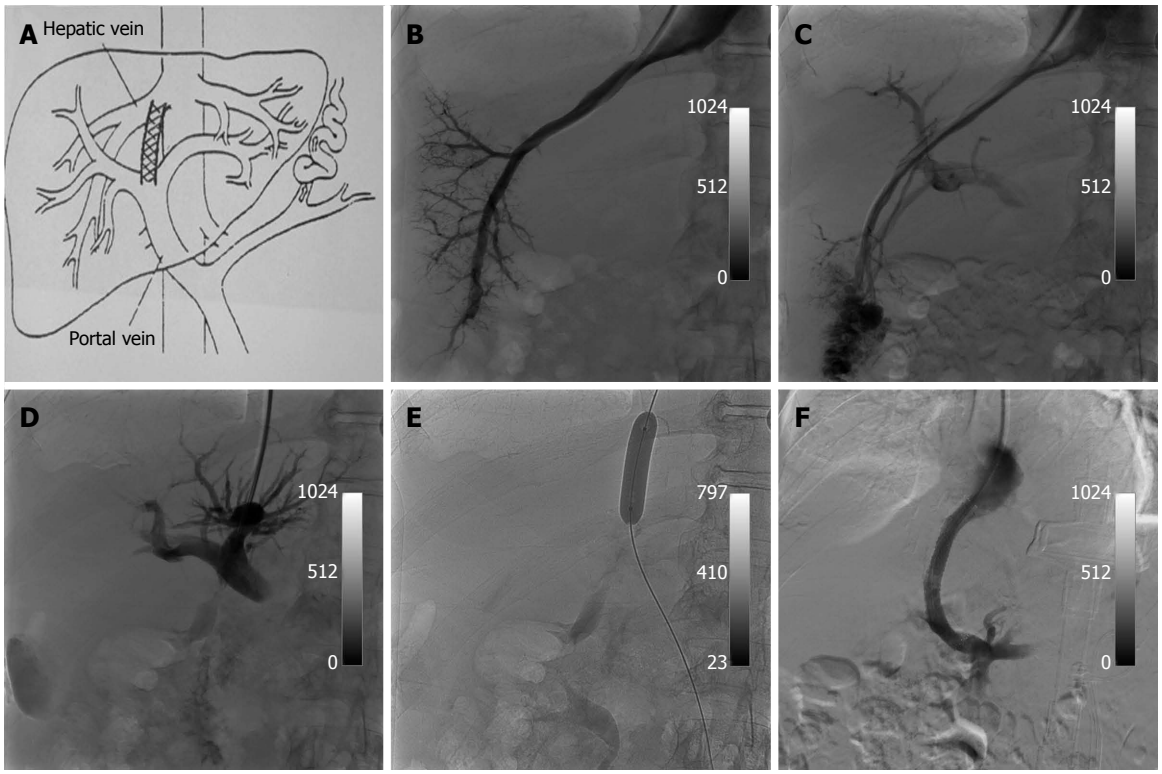

Figure 1 Conventional transjugular intrahepatic portosystemic shunt creation technique. A: Schematic diagram shows transjugular intrahepatic portosystemic shunt (TIPS) connecting the right hepatic vein to the right portal vein. The shunt extends from main portal vein to confluence of right hepatic vein and inferior vena cava; B: Right hepatic venogram shows course of hepatic vein; C: Transhepatic portogram using iodinated contrast material shows course of portal veins; D: Injection of contrast medium through Colapinto needle confirms needle position within portal vein before passage of guidewire; E: Dilatation of a tract through the hepatic parenchyma that is interposed between the hepatic and portal veins; F: Portal venogram obtained after TIPS insertion shows flow through the FLUENCY polytetrafluoroethylene-covered stent. Peripheral portal vein branches are no longer opacified because of reversal of flow.

treatment to prevent hemoperitoneum. The TIPS tract must be intraparenchymal, or dilatation of the extrahepatic portion of the portal vein results in fast exsanguination, a complication that occurs in approximately 1% of procedures. Entry into the right or left portal vein branch should be at least 1 to 2 cm from the portal vein bifurcation. The direct injection into the dilated tract should be done as soon as possible to reveal potential extravasation. If it is positive, the balloon is again inflated and the stent graft placed to tamponade the extrahepatic leak. According to the patient's blood pressure, fluid volume resuscitation is immediately initiated and the anesthesiologist is called[6,9,50,66]. The final step of the TIPS procedure is placement of pigtail catheter over the portal vein guide wire for follow-up portography and blood pressure measurement within the main portal vein. Once the value is stabilized and recorded, the tip of the sheath or pigtail catheter is moved to the hepatic vein or the suprahepatic inferior vena cava, and the blood pressure is again recorded. Thus, at the completion of the TIPS procedure, at least four pressure values will have been obtained: those in the portal vein and hepatic vein (or inferior vena cava) before and after shunt placement. The different steps are summarized in Figure 1.

Embolization of varices

Embolization of the esophageal varices at the time of the TIPS is easily accomplished but its routine application has been also controversial. While embolization after TIPS occurs in 24% to 48% of patients[68,69], it is not clear whether the combination of TIPS and variceal embolization is more effective than TIPS alone. Some authors recommend transjugular embolization of the varices to increase the effect of the shunt with respect to acute hemostasis[68,70], and other authors do not perform embolization[71]. In our clinical practice, we perform embolization of varices only if we observe persistent contrast flow into the varices in the control portography after TIPS. Variceal embolization is also indicated for patients with recurrent esophageal bleeding despite a patent shunt[68]. Embolization of esophageal varices is most commonly performed with the use of metallic coils, but the use of liquid agents such as opacified enbucrilate and ethanol have also been described[17]. The use of absolute ethanol is not recommended due to the possible adverse effects including cardiovascular collapse due to the possible venous channels between the portal system and the pericardium, mainly from the pericardiophrenic vein.

Post-procedural follow-up

Recurrence or worsening of the portal hypertension symptoms should prompt an ultrasound with Doppler to exclude TIPS stenosis. Shunt velocities between 50 and 250 cm/s are associated with high (> 90%) sensitiv-

Table 3 Acute and chronic complications after transjugular intrahepatic portosystemic shunt placement

Acute complications	Acute complications	Chronic complications
Minor or moderate	Life-threatening	Portal vein thrombosis
Stent displacement	Hemobilia	Congestive heart failure
Neck hematoma	Hemoperitoneum	Progressive liver failure
Arrhythmia	Cardiac failure	Chronic recurrent encephalopathy
Shunt thrombosis	Liver ischemia	Stent dysfunction
Hepatic vein obstruction	Sepsis	

Table 4 Risk factors for post-transjugular intrahepatic portosystemic shunt encephalopathy

Risk factors
Age
Sex
Cause
Child-Pugh score
Hepatic encephalopathy history TIPS
Porto-hepatic gradient
Stent diameter
Indication
Creatinine

TIPS: Transjugular intrahepatic portosystemic shunt.

ity and specificity for shunt dysfunction[72]. In addition, most hepatologists order routine TIPS surveillance tests at regular intervals using ultrasound with Doppler in asymptomatic patients. Patients with a suspected TIPS dysfunction should undergo TIPS venography. If the original TIPS was created using a bare-metal stent, placement of a covered stent is likely to improve long-term shunt patency[73]. Other commonly used measures include balloon angioplasty within the stents and the placement of additional stents in patients to extend cranial or caudal length of the stent. Hepatic encephalopathy refractory to medical management or progressive hepatic dysfunction after TIPS placement might require endovascular shunt reduction. A commonly used technique involves shunt catheterization by two parallel guidewires followed by simultaneous deployment of two stents within the shunt. One of the stents is a covered endograft through which blood flow will be conducted, whereas the second device is a balloon-expandable bare-metal stent, the diameter of which determines the ultimate shunt diameter. Usually, the bare-metal stent is placed along the cephalic aspect of the covered stent. This allows continued access to the balloon expandable stent if further reduction is necessary[74,75].

OUTCOMES

Complications

The TIPS procedure may lead to a number of adverse events (Table 3). Technical complications sustained at the time of TIPS placement can include transcapsular puncture, which may occur in as many as 33% of cases[10]. The capsular perforation leads to significant intraperitoneal hemorrhage 1% to 2% of the time[10]. Clinically significant hemobilia is also rarely observed after the procedure. The stent can be placed too far into the inferior vena cava or even into the right atrium at the cranial end or far into the main portal vein at the caudal end of the shunt in up to 20% of patients[10]. On occasion, stents may migrate because of catheter and balloon manipulation[76]. Diversion of portal venous flow through the shunt diminishes the metabolic filtering effect of the hepatic parenchyma, leading to new or worsened encephalopathy in 30% to 46% of patients[34,35]. Chronic recurrent disabling hepatic encephalopathy can occur in 5% to 10% of patients and may lead to a complete loss of the patient's autonomy[10,66]. Several pre-TIPS parameters have been tested to predict post-TIPS hepatic encephalopathy (Table 4)[10,34,35]. Deterioration of hepatic function in approximately 10% of patients[35], and hepatorenal syndrome is occasionally observed[77]. TIPS stenosis and occlusion was the method of choice before wide acceptance of PTFE-covered stents (Viatorr; W.L. Gore and Associates, Flagstaff, AZ, United States). The most common site of shunt stenosis is at the hepatic venous end. The culprit of midstent stenosis is thought to be intimal hyperplasia within the bare-metal stent due to contact between traversed biliary radicles and stent lumen[76]. The incidence of stenosis due to hyperplasia within the stent ranged from 18% to 78%[76] for bare-metal stents, which led to recurrence of portal hypertension complications and required frequent invasive procedures for reconstitution of flow. The introduction of PTFE-covered stent grafts led to dramatic improvement in long-term TIPS patency. A randomized controlled trial published in 2007 established a PTFE-covered stent as the preferred device for TIPS[78]. In that study, 80 patients were randomized to receive either a covered (n = 39) or a bare (n = 41) metal stent and were followed for two years after TIPS placement. Compared with patients treated with a bare-metal stent, patients with a PTFE-covered stent had a significantly lower rate of TIPS dysfunction (15% *vs* 44%), a higher rate of primary patency (76% *vs* 36%), a lower rate of clinical relapse (10% *vs* 29%), and were less likely to develop encephalopathy (33% *vs* 49%)[78]. On the basis of these data, a PTFE-covered stent became the standard of care device for de novo TIPS. Patients who have a bare metal stent TIPS should undergo shunt revision with a PTFE-covered stent in the event of shunt dysfunction[76].

Mortality

Acute variceal hemorrhage that is poorly controlled with endoscopic therapy is generally well controlled with TIPS, which has a success rate of 90% to 100%. However, TIPS also has a mortality rate of 27% to 50%[19,66,79,80]. Increased mortality is related to a Child-Pugh C clinical status, hemodynamic instability at the time of the TIPS procedure, and the presence of other comorbidities. In general, early TIPS intervention allows for better control of hemorrhage with decreased mortality. Patients with a

high HVPG (> 20 mmHg) and acute variceal bleeding have a better survival with TIPS than with endoscopic therapy[65]. Most of the deaths of patients after emergency TIPS are related to hepatic failure, multiorgan failure, and sepsis, often accompanied by variceal and nonvariceal bleeding, while only a minority are related to recurrent variceal bleeding[13,69,81]. Death occurring within 30 d of the procedure is most commonly caused by multiorgan failure, and death more than 30 d following the procedure is most commonly related to liver failure[81]. Many studies reporting on emergency TIPS for the rescue treatment of acute esophageal varices bleeding have shown low survival rates and significantly higher mortality rates than patients undergoing elective TIPS[6,12,17,19,21,65]. In one study, 42 of 123 (34.1%) of patients died within 30 d of TIPS for acute bleeding, while only 16.5% died following elective TIPS creation[82]. As an independent predictor of mortality, patients bleeding at the time of TIPS creation were 2.9 times more likely to die than those associated with elective TIPS placement. Similar findings have been reported by Helton *et al*[83] who reported a 56% in-hospital mortality rate for patients who were actively bleeding or hemodynamically unstable at the time of the TIPS *vs* 5.5% following nonemergency procedures. The reported mortality associated with TIPS varies widely because the inclusion criteria, timing of the TIPS, and the severity of liver disease. Many reports combine the results of patients actively bleeding during TIPS with those of the patients who were stable during the procedure. Several reports describing different prognostic factors associated with mortality after TIPS have been published[68,69,82]. Prognostic factors are not intended to predict outcome or management on individual basis or to deny a patient a potentially lifesaving intervention, but are useful as guidelines to develop appropriate expectations and to weigh different therapeutic options. Final decisions are based on the individual patient needs and overall clinical condition[65,84]. Many of these prognostic factors correlate with the mortality of patients undergoing elective TIPS. In patients with acute variceal bleeding, however, these predictors may fail because the hepatic reserve and renal function are difficult to evaluate in the acute setting[65]. Events such as bleeding, infection, and high-dose diuretic therapy may affect the renal and liver function in a transient way. No single prognostic criterion is available to accurately select patients with a very high risk of death[85]. However, several selection criteria have been described due to an increased amount of experience within the field with relation to TIPS[86].

Effect on liver and spleen stiffness

Variceal bleeding still remains the major cause of death in patients with cirrhosis, with increasing numbers of inpatient cases with advanced liver disease and portal hypertension. For those patients, TIPS has become the rescue treatment of choice, preferred over liver transplantation. Therefore, it is crucial to ensure that the inserted TIPS effectively decreases portal vein pressure to prevent variceal bleeding. Non-invasively assessing the pressure of the portal vein as a function of the TIPS has been a challenge. Color Doppler sonography can measure flow velocities in the TIPS, but it cannot reflect the pressure of the portal vein and its pitfalls and inaccuracies lead to a lack in necessary sensitivity[87]. More recently, a novel ultrasound-based acoustic radiation force impulse (ARFI) elastography has been developed that can provide information on the local mechanical property of a tissue[88]. An acoustic push pulse transmitted by the transducer toward the tissue produces an elastic shear wave that propagates through the tissue. The propagation of the shear wave is followed by detection pulses that are used to measure the velocity of the shear wave propagation, which is directly related to tissue elasticity. In other words, the speed of shear wave is dependent on the elasticity of the tissue[88].

Gao *et al*[89] prospectively assessed the stiffness of the liver and spleen with ARFI imaging pre- and post-TIPS placement. The investigators measured stiffness of the liver and spleen with mean shear wave velocity (MSV, m/s) on ARFI imaging for 10 healthy volunteers and 10 patients who underwent TIPS placement for treatment of portal hypertension. The portal vein pressure was measured during TIPS placement. A significant difference in portal vein pressure was found for the pre- (27.67 ± 5.86 mmHg) and post- (18.00 ± 6.93 mmHg) TIPS insertion. Significant differences were also found in MSV of the liver and spleen between healthy subjects and patients with portal hypertension. There was no significant difference found in MSV of the liver pre- and post-TIPS placement. However, a statistically significant difference in MSV of the spleen pre- and post-TIPS placement was demonstrated. In addition, the authors reported a significant difference in spleen index between healthy subjects and patients with portal hypertension, as well as between pre- and post-TIPS placement. The MSV of the spleen measured with ARFI correlated well with portal vein pressure. Hence, the authors concluded that spleen stiffness determined by means of MSV on ARFI imaging could be used as a quantitative marker for monitoring the portal vein pressure as the function of the TIPS.

In this study, as well, the authors had prospectively shown a close correlation between the stiffness of the spleen and portal vein pressure. Based on these data, one can clearly note that the stiffness of the spleen measured with MSV changes as the portal vein pressure changes following TIPS placement. This is the first quantitative demonstration of the effectiveness of TIPS on the stiffness of the spleen measured with MSV value on ARFI imaging. One parameter that was not significantly affected by TIPS placement was the MSV value of the liver. The most plausible explanation for this finding is that TIPS can have a direct impact on the pressure of the portal vein but have no effect on the stiffness of a fibrotic liver. The tissue mechanical property of a cirrhotic liver is very hard due to the severe fibrosis developed in the liver parenchyma, which has poor elasticity. In addition, MSV of the spleen has potential to serve as an indicating

marker with which to assess portal vein pressure. Finally, it may be used as a non-invasive predictor in screening for recurrent portal hypertension when TIPS malfunction develops.

Economic benefit

Early insertion of TIPS in high-risk patients with acute variceal hemorrhage reduces rebleeding and mortality. However, the economic benefit of utilizing this approach remains unclear. Harman *et al*[90] retrospectively carried out a cost-effectiveness analysis of patients who may benefit from early TIPS insertion. The costs were calculated in a 12-mo follow-up from index bleeding admission and compared to a theoretical 12-mo follow-up cost related to early TIPS insertion. Over one year, 78 patients were admitted with variceal hemorrhage; 27 patients (35%) were eligible for early TIPS insertion. The actual cost for the 12-mo follow-up was £138473.50. The authors estimated early TIPS insertion would save £534.70 per patient per year ($P < 0.0001$). According to sensitivity analysis, early TIPS was the dominant treatment modality up to a theoretical rebleeding rate of 6%, and the economic threshold of £15000 per bleeding episode saved was achieved at a 12% yearly rebleeding rate, suggesting it would be financially viable to adopt early TIPS as an intervention up to a 12% yearly rebleeding rate. This study indicates strict patient selection is vital to reduce the rebleeding rate when utilizing early TIPS insertion. There is an important balance between selecting patients at high risk of rebleeding, who are likely to benefit from early TIPS insertion to prevent rebleeding, but also to exclude patients with the most severe hepatic dysfunction where early TIPS insertion is unlikely to alter the natural history of their disease. Strict patient selection reduces rebleeding-related admissions, thus reducing follow-up costs; this is a key concept for centers to focus on before introducing early TIPS as routine practice. Finally, Harman *et al*[90] found 35% of their bleeding cohort were eligible for early TIPS insertion, further establishing early TIPS insertion as a cost-effective intervention. This has important implications for the future provision and organization of interventional radiology services. Future prospective studies evaluating early TIPS insertion are warranted, and including similar economic modeling will help to confirm the financial viability of introducing early TIPS insertion into routine clinical practice.

CONCLUSION

The TIPS procedure is now a well-established treatment for complications of portal hypertension. Technical advances and well-designed clinical studies provide a scientific basis to define the best indications. Patients with acute variceal bleeding with a Child-Pugh score > 12, APACHE score Ⅱ > 18 points, hemodynamically unstable, receiving vasopressors and coagulopathy, and/or bilirubin > 6 mg/dL have a high risk of early death after TIPS. In specific, in some individual clinical situations it may be wise to withhold the TIPS because the mortality rate will be very high regardless of the therapy given. Every effort should be taken to stabilize the patient before TIPS, including the use of tamponade tubes and aggressive correction of coagulopathy. Once medical treatment and sclerotherapy fail, emergency TIPS should be considered early before the clinical condition worsens. Patients at high risk for early mortality after TIPS should be considered for expedited liver transplantation if available. Cost analysis must be performed in the future taking into account recent developments including technical improvements, better patient selection, and better post-TIPS management.

REFERENCES

1 **Jalan R**, Hayes PC. UK guidelines on the management of variceal haemorrhage in cirrhotic patients. British Society of Gastroenterology. *Gut* 2000; **46** Suppl 3-4: III1-III15 [PMID: 10862604]

2 **North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices**. Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A prospective multicenter study. *N Engl J Med* 1988; **319**: 983-989 [PMID: 3262200 DOI: 10.1056/NEJM198810133191505]

3 **D'Amico G**, Pagliaro L, Bosch J. The treatment of portal hypertension: a meta-analytic review. *Hepatology* 1995; **22**: 332-354 [PMID: 7601427 DOI: 10.1002/hep.1840220145]

4 **de Dombal FT**, Clarke JR, Clamp SE, Malizia G, Kotwal MR, Morgan AG. Prognostic factors in upper G.I. bleeding. *Endoscopy* 1986; **18** Suppl 2: 6-10 [PMID: 3519197 DOI: 10.1055/s-2007-1018418]

5 **Gores GJ**, Wiesner RH, Dickson ER, Zinsmeister AR, Jorgensen RA, Langworthy A. Prospective evaluation of esophageal varices in primary biliary cirrhosis: development, natural history, and influence on survival. *Gastroenterology* 1989; **96**: 1552-1559 [PMID: 2785470]

6 **McAvoy NC**, Hayes PC. The use of transjugular intrahepatic portosystemic stent shunt in the management of acute oesophageal variceal haemorrhage. *Eur J Gastroenterol Hepatol* 2006; **18**: 1135-1141 [PMID: 17033431 DOI: 10.1097/01.meg.0000236877.98472.58]

7 **Moitinho E**, Escorsell A, Bandi JC, Salmerón JM, García-Pagán JC, Rodés J, Bosch J. Prognostic value of early measurements of portal pressure in acute variceal bleeding. *Gastroenterology* 1999; **117**: 626-631 [PMID: 10464138 DOI: 10.1016/S0016-5085(99)70455-5]

8 **Beppu K**, Inokuchi K, Koyanagi N, Nakayama S, Sakata H, Kitano S, Kobayashi M. Prediction of variceal hemorrhage by esophageal endoscopy. *Gastrointest Endosc* 1981; **27**: 213-218 [PMID: 6975734 DOI: 10.1016/S0016-5107(81)73224-3]

9 **March RE**, Kirwan JR, Davies PG, Winrow VR, Holborow EJ. Sensitive ELISA measurement of IgA, IgG and IgM antiglobulins in rheumatic and other diseases and a comparison of their specificity in rheumatoid arthritis and infectious mononucleosis. *Scand J Rheumatol* 1987; **16**: 445-449 [PMID: 3501161 DOI: 10.1007/s00270-012-0493-y]

10 **Pomier-Layrargues G**, Bouchard L, Lafortune M, Bissonnette J, Guérette D, Perreault P. The transjugular intrahepatic portosystemic shunt in the treatment of portal hypertension: current status. *Int J Hepatol* 2012; **2012**: 167868 [PMID: 22888442]

11 **Irani S**, Kowdley K, Kozarek R. Gastric varices: an updated review of management. *J Clin Gastroenterol* 2011; **45**: 133-148 [PMID: 21135706 DOI: 10.1097/MCG.0b013e3181fbe249]

12 **Barange K**, Péron JM, Imani K, Otal P, Payen JL, Rousseau H, Pascal JP, Joffre F, Vinel JP. Transjugular intrahepatic portosystemic shunt in the treatment of refractory bleeding from ruptured gastric varices. *Hepatology* 1999; **30**: 1139-1143 [PMID: 10534333 DOI: 10.1002/hep.510300523]

13 **Chau TN**, Patch D, Chan YW, Nagral A, Dick R, Burroughs AK. "Salvage" transjugular intrahepatic portosystemic shunts: gastric fundal compared with esophageal variceal bleeding. *Gastroenterology* 1998; **114**: 981-987 [PMID: 9558287 DOI: 10.1016/S0016-5085(98)00640-4]

14 **Lo GH**, Liang HL, Chen WC, Chen MH, Lai KH, Hsu PI, Lin CK, Chan HH, Pan HB. A prospective, randomized controlled trial of transjugular intrahepatic portosystemic shunt versus cyanoacrylate injection in the prevention of gastric variceal rebleeding. *Endoscopy* 2007; **39**: 679-685 [PMID: 17661241 DOI: 10.1055/s-2007-966591]

15 **Tripathi D**, Therapondos G, Jackson E, Redhead DN, Hayes PC. The role of the transjugular intrahepatic portosystemic stent shunt (TIPSS) in the management of bleeding gastric varices: clinical and haemodynamic correlations. *Gut* 2002; **51**: 270-274 [PMID: 12117893 DOI: 10.1136/gut.51.2.270]

16 **Tesdal IK**, Filser T, Weiss C, Holm E, Dueber C, Jaschke W. Transjugular intrahepatic portosystemic shunts: adjunctive embolotherapy of gastroesophageal collateral vessels in the prevention of variceal rebleeding. *Radiology* 2005; **236**: 360-367 [PMID: 15955858 DOI: 10.1148/radiol.2361040530]

17 **Azoulay D**, Castaing D, Majno P, Saliba F, Ichaï P, Smail A, Delvart V, Danaoui M, Samuel D, Bismuth H. Salvage transjugular intrahepatic portosystemic shunt for uncontrolled variceal bleeding in patients with decompensated cirrhosis. *J Hepatol* 2001; **35**: 590-597 [PMID: 11690704]

18 **D'Amico G**, Luca A. TIPS is a cost effective alternative to surgical shunt as a rescue therapy for prevention of recurrent bleeding from esophageal varices. *J Hepatol* 2008; **48**: 387-390 [PMID: 18199520 DOI: 10.1016/j.jhep.2007.12.009]

19 **Sanyal AJ**, Freedman AM, Luketic VA, Purdum PP, Shiffman ML, Tisnado J, Cole PE. Transjugular intrahepatic portosystemic shunts for patients with active variceal hemorrhage unresponsive to sclerotherapy. *Gastroenterology* 1996; **111**: 138-146 [PMID: 8698192 DOI: 10.1053/gast.1996.v111.pm8698192]

20 **Vangeli M**, Patch D, Burroughs AK. Salvage tips for uncontrolled variceal bleeding. *J Hepatol* 2002; **37**: 703-704 [PMID: 12399244 DOI: 10.1016/S0168-8278(02)00321-5]

21 **García-Pagán JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A, Abraldes JG, Nevens F, Vinel JP, Mössner J, Bosch J. Early use of TIPS in patients with cirrhosis and variceal bleeding. *N Engl J Med* 2010; **362**: 2370-2379 [PMID: 20573925 DOI: 10.1056/NEJMoa0910102]

22 **Rössle M**, Haag K, Ochs A, Sellinger M, Nöldge G, Perarnau JM, Berger E, Blum U, Gabelmann A, Hauenstein K. The transjugular intrahepatic portosystemic stent-shunt procedure for variceal bleeding. *N Engl J Med* 1994; **330**: 165-171 [PMID: 8264738 DOI: 10.1056/NEJM199401203300303]

23 **Cello JP**, Ring EJ, Olcott EW, Koch J, Gordon R, Sandhu J, Morgan DR, Ostroff JW, Rockey DC, Bacchetti P, LaBerge J, Lake JR, Somberg K, Doherty C, Davila M, McQuaid K, Wall SD. Endoscopic sclerotherapy compared with percutaneous transjugular intrahepatic portosystemic shunt after initial sclerotherapy in patients with acute variceal hemorrhage. A randomized, controlled trial. *Ann Intern Med* 1997; **126**: 858-865 [PMID: 9163286 DOI: 10.7326/0003-4819-126-11-199706010-00002]

24 **Sanyal AJ**, Freedman AM, Luketic VA, Purdum PP, Shiffman ML, Cole PE, Tisnado J, Simmons S. Transjugular intrahepatic portosystemic shunts compared with endoscopic sclerotherapy for the prevention of recurrent variceal hemorrhage. A randomized, controlled trial. *Ann Intern Med* 1997; **126**: 849-857 [PMID: 9163285 DOI: 10.7326/0003-4819-126-11-199706010-00001]

25 **Cabrera J**, Maynar M, Granados R, Gorriz E, Reyes R, Pulido-Duque JM, Rodriguez SanRoman JL, Guerra C, Kravetz D. Transjugular intrahepatic portosystemic shunt versus sclerotherapy in the elective treatment of variceal hemorrhage. *Gastroenterology* 1996; **110**: 832-839 [PMID: 8608893 DOI: 10.1053/gast.1996.v110.pm8608893]

26 **Sauer P**, Theilmann L, Stremmel W, Benz C, Richter GM, Stiehl A. Transjugular intrahepatic portosystemic stent shunt versus sclerotherapy plus propranolol for variceal rebleeding. *Gastroenterology* 1997; **113**: 1623-1631 [PMID: 9352865 DOI: 10.1053/gast.1997.v113.pm9352865]

27 **Jalan R**, Forrest EH, Stanley AJ, Redhead DN, Forbes J, Dillon JF, MacGilchrist AJ, Finlayson ND, Hayes PC. A randomized trial comparing transjugular intrahepatic portosystemic stent-shunt with variceal band ligation in the prevention of rebleeding from esophageal varices. *Hepatology* 1997; **26**: 1115-1122 [PMID: 9362350 DOI: 10.1002/hep.510260505]

28 **Merli M**, Salerno F, Riggio O, de Franchis R, Fiaccadori F, Meddi P, Primignani M, Pedretti G, Maggi A, Capocaccia L, Lovaria A, Ugolotti U, Salvatori F, Bezzi M, Rossi P. Transjugular intrahepatic portosystemic shunt versus endoscopic sclerotherapy for the prevention of variceal bleeding in cirrhosis: a randomized multicenter trial. Gruppo Italiano Studio TIPS (G.I.S.T.). *Hepatology* 1998; **27**: 48-53 [PMID: 9425916 DOI: 10.1002/hep.510270109]

29 **Rössle M**, Deibert P, Haag K, Ochs A, Olschewski M, Siegerstetter V, Hauenstein KH, Geiger R, Stiepak C, Keller W, Blum HE. Randomised trial of transjugular-intrahepatic-portosystemic shunt versus endoscopy plus propranolol for prevention of variceal rebleeding. *Lancet* 1997; **349**: 1043-1049 [PMID: 9107241]

30 **García-Villarreal L**, Martínez-Lagares F, Sierra A, Guevara C, Marrero JM, Jiménez E, Monescillo A, Hernández-Cabrero T, Alonso JM, Fuentes R. Transjugular intrahepatic portosystemic shunt versus endoscopic sclerotherapy for the prevention of variceal rebleeding after recent variceal hemorrhage. *Hepatology* 1999; **29**: 27-32 [PMID: 9862845 DOI: 10.1002/hep.510290125]

31 **Pomier-Layrargues G**, Villeneuve JP, Deschênes M, Bui B, Perreault P, Fenyves D, Willems B, Marleau D, Bilodeau M, Lafortune M, Dufresne MP. Transjugular intrahepatic portosystemic shunt (TIPS) versus endoscopic variceal ligation in the prevention of variceal rebleeding in patients with cirrhosis: a randomised trial. *Gut* 2001; **48**: 390-396 [PMID: 11171831 DOI: 10.1136/gut.48.3.390]

32 **Narahara Y**, Kanazawa H, Kawamata H, Tada N, Saitoh H, Matsuzaka S, Osada Y, Mamiya Y, Nakatsuka K, Yoshimoto H, Koizumi N, Sakamoto C, Kobayashi M. A randomized clinical trial comparing transjugular intrahepatic portosystemic shunt with endoscopic sclerotherapy in the long-term management of patients with cirrhosis after recent variceal hemorrhage. *Hepatol Res* 2001; **21**: 189-198 [PMID: 11673103 DOI: 10.1016/S1386-6346(01)00104-8]

33 **Gülberg V**, Schepke M, Geigenberger G, Holl J, Brensing KA, Waggershauser T, Reiser M, Schild HH, Sauerbruch T, Gerbes AL. Transjugular intrahepatic portosystemic shunting is not superior to endoscopic variceal band ligation for prevention of variceal rebleeding in cirrhotic patients: a randomized, controlled trial. *Scand J Gastroenterol* 2002; **37**: 338-343 [PMID: 11916197 DOI: 10.1080/003655202317284255]

34 **Burroughs AK**, Vangeli M. Transjugular intrahepatic portosystemic shunt versus endoscopic therapy: randomized trials for secondary prophylaxis of variceal bleeding: an updated meta-analysis. *Scand J Gastroenterol* 2002; **37**: 249-252 [PMID: 11916185 DOI: 10.1080/003655202317284138]

35 **Zheng M**, Chen Y, Bai J, Zeng Q, You J, Jin R, Zhou X, Shen H, Zheng Y, Du Z. Transjugular intrahepatic portosystemic shunt versus endoscopic therapy in the secondary prophylaxis of variceal rebleeding in cirrhotic patients: meta-analysis update. *J Clin Gastroenterol* 2008; **42**: 507-516 [PMID:

18344888 DOI: 10.1097/MCG.0b013e31815576e6]
36 **Vangeli M**, Patch D, Terreni N, Tibballs J, Watkinson A, Davies N, Burroughs AK. Bleeding ectopic varices--treatment with transjugular intrahepatic porto-systemic shunt (TIPS) and embolisation. *J Hepatol* 2004; **41**: 560-566 [PMID: 15464235 DOI: 10.1016/j.jhep.2004.06.024]
37 **Vidal V**, Joly L, Perreault P, Bouchard L, Lafortune M, Pomier-Layrargues G. Usefulness of transjugular intrahepatic portosystemic shunt in the management of bleeding ectopic varices in cirrhotic patients. *Cardiovasc Intervent Radiol* 2006; **29**: 216-219 [PMID: 16284702 DOI: 10.1007/s00270-004-0346-4]
38 **Kamath PS**, Lacerda M, Ahlquist DA, McKusick MA, Andrews JC, Nagorney DA. Gastric mucosal responses to intrahepatic portosystemic shunting in patients with cirrhosis. *Gastroenterology* 2000; **118**: 905-911 [PMID: 10784589 DOI: 10.1016/S0016-5085(00)70176-4]
39 **Spahr L**, Villeneuve JP, Dufresne MP, Tassé D, Bui B, Willems B, Fenyves D, Pomier-Layrargues G. Gastric antral vascular ectasia in cirrhotic patients: absence of relation with portal hypertension. *Gut* 1999; **44**: 739-742 [PMID: 10205216 DOI: 10.1136/gut.44.5.739]
40 **Garcia-Pagán JC**, Heydtmann M, Raffa S, Plessier A, Murad S, Fabris F, Vizzini G, Gonzales Abraldes J, Olliff S, Nicolini A, Luca A, Primignani M, Janssen HL, Valla D, Elias E, Bosch J. TIPS for Budd-Chiari syndrome: long-term results and prognostics factors in 124 patients. *Gastroenterology* 2008; **135**: 808-815 [PMID: 18621047 DOI: 10.1053/j.gastro.2008.05.051]
41 **Hernández-Guerra M**, Turnes J, Rubinstein P, Olliff S, Elias E, Bosch J, García-Pagán JC. PTFE-covered stents improve TIPS patency in Budd-Chiari syndrome. *Hepatology* 2004; **40**: 1197-1202 [PMID: 15486923 DOI: 10.1002/hep.20436]
42 **Azoulay D**, Castaing D, Lemoine A, Hargreaves GM, Bismuth H. Transjugular intrahepatic portosystemic shunt (TIPS) for severe veno-occlusive disease of the liver following bone marrow transplantation. *Bone Marrow Transplant* 2000; **25**: 987-992 [PMID: 10800068 DOI: 10.1038/sj.bmt.1702386]
43 **Dhanasekaran R**, West JK, Gonzales PC, Subramanian R, Parekh S, Spivey JR, Martin LG, Kim HS. Transjugular intrahepatic portosystemic shunt for symptomatic refractory hepatic hydrothorax in patients with cirrhosis. *Am J Gastroenterol* 2010; **105**: 635-641 [PMID: 19904245 DOI: 10.1038/ajg.2009.634]
44 **Siegerstetter V**, Deibert P, Ochs A, Olschewski M, Blum HE, Rössle M. Treatment of refractory hepatic hydrothorax with transjugular intrahepatic portosystemic shunt: long-term results in 40 patients. *Eur J Gastroenterol Hepatol* 2001; **13**: 529-534 [PMID: 11396532 DOI: 10.1097/00042737-200105000-00011]
45 **Wilputte JY**, Goffette P, Zech F, Godoy-Gepert A, Geubel A. The outcome after transjugular intrahepatic portosystemic shunt (TIPS) for hepatic hydrothorax is closely related to liver dysfunction: a long-term study in 28 patients. *Acta Gastroenterol Belg* 2007; **70**: 6-10 [PMID: 17619531]
46 **Spencer EB**, Cohen DT, Darcy MD. Safety and efficacy of transjugular intrahepatic portosystemic shunt creation for the treatment of hepatic hydrothorax. *J Vasc Interv Radiol* 2002; **13**: 385-390 [PMID: 11932369 DOI: 10.1016/S1051-0443(07)61741-2]
47 **Brensing KA**, Textor J, Perz J, Schiedermaier P, Raab P, Strunk H, Klehr HU, Kramer HJ, Spengler U, Schild H, Sauerbruch T. Long term outcome after transjugular intrahepatic portosystemic stent-shunt in non-transplant cirrhotics with hepatorenal syndrome: a phase II study. *Gut* 2000; **47**: 288-295 [PMID: 10896924 DOI: 10.1136/gut.47.2.288]
48 **Testino G**, Ferro C, Sumberaz A, Messa P, Morelli N, Guadagni B, Ardizzone G, Valente U. Type-2 hepatorenal syndrome and refractory ascites: role of transjugular intrahepatic portosystemic stent-shunt in eighteen patients with advanced cirrhosis awaiting orthotopic liver transplantation. *Hepatogastroenterology* 2003; **50**: 1753-1755 [PMID: 14696397]
49 **Martinez-Palli G**, Drake BB, Garcia-Pagan JC, Barbera JA, Arguedas MR, Rodriguez-Roisin R, Bosch J, Fallon MB. Effect of transjugular intrahepatic portosystemic shunt on pulmonary gas exchange in patients with portal hypertension and hepatopulmonary syndrome. *World J Gastroenterol* 2005; **11**: 6858-6862 [PMID: 16425397]
50 **Fidelman N**, Kwan SW, LaBerge JM, Gordon RL, Ring EJ, Kerlan RK. The transjugular intrahepatic portosystemic shunt: an update. *AJR Am J Roentgenol* 2012; **199**: 746-755 [PMID: 22997364 DOI: 10.2214/AJR.12.9101]
51 **Kravetz D**, Bosch J, Arderiu M, Pilar Pizcueta M, Rodés J. Hemodynamic effects of blood volume restitution following a hemorrhage in rats with portal hypertension due to cirrhosis of the liver: influence of the extent of portal-systemic shunting. *Hepatology* 1989; **9**: 808-814 [PMID: 2714733 DOI: 10.1002/hep.1840090603]
52 **Bernard B**, Cadranel JF, Valla D, Escolano S, Jarlier V, Opolon P. Prognostic significance of bacterial infection in bleeding cirrhotic patients: a prospective study. *Gastroenterology* 1995; **108**: 1828-1834 [PMID: 7768389 DOI: 10.1016/0016-5085(95)90146-9]
53 **Goulis J**, Armonis A, Patch D, Sabin C, Greenslade L, Burroughs AK. Bacterial infection is independently associated with failure to control bleeding in cirrhotic patients with gastrointestinal hemorrhage. *Hepatology* 1998; **27**: 1207-1212 [PMID: 9581672 DOI: 10.1002/hep.510270504]
54 **Bernard B**, Grangé JD, Khac EN, Amiot X, Opolon P, Poynard T. Antibiotic prophylaxis for the prevention of bacterial infections in cirrhotic patients with gastrointestinal bleeding: a meta-analysis. *Hepatology* 1999; **29**: 1655-1661 [PMID: 10347104 DOI: 10.1002/hep.510290608]
55 **Blaise M**, Pateron D, Trinchet JC, Levacher S, Beaugrand M, Pourriat JL. Systemic antibiotic therapy prevents bacterial infection in cirrhotic patients with gastrointestinal hemorrhage. *Hepatology* 1994; **20**: 34-38 [PMID: 8020902 DOI: 10.1002/hep.1840200107]
56 **Selby WS**, Norton ID, Pokorny CS, Benn RA. Bacteremia and bacterascites after endoscopic sclerotherapy for bleeding esophageal varices and prevention by intravenous cefotaxime: a randomized trial. *Gastrointest Endosc* 1994; **40**: 680-684 [PMID: 7859964]
57 **Hsieh WJ**, Lin HC, Hwang SJ, Hou MC, Lee FY, Chang FY, Lee SD. The effect of ciprofloxacin in the prevention of bacterial infection in patients with cirrhosis after upper gastrointestinal bleeding. *Am J Gastroenterol* 1998; **93**: 962-966 [PMID: 9647029 DOI: 10.1111/j.1572-0241.1998.00288.x]
58 **Young MF**, Sanowski RA, Rasche R. Comparison and characterization of ulcerations induced by endoscopic ligation of esophageal varices versus endoscopic sclerotherapy. *Gastrointest Endosc* 1993; **39**: 119-122 [PMID: 8495829 DOI: 10.1016/S0016-5107(93)70049-8]
59 **Levacher S**, Letoumelin P, Pateron D, Blaise M, Lapandry C, Pourriat JL. Early administration of terlipressin plus glyceryl trinitrate to control active upper gastrointestinal bleeding in cirrhotic patients. *Lancet* 1995; **346**: 865-868 [PMID: 7564670 DOI: 10.1016/S0140-6736(95)92708-5]
60 **Avgerinos A**, Nevens F, Raptis S, Fevery J. Early administration of somatostatin and efficacy of sclerotherapy in acute oesophageal variceal bleeds: the European Acute Bleeding Oesophageal Variceal Episodes (ABOVE) randomised trial. *Lancet* 1997; **350**: 1495-1499 [PMID: 9388396 DOI: 10.1016/S0140-6736(97)05099-X]
61 **Calès P**, Masliah C, Bernard B, Garnier PP, Silvain C, Szostak-Talbodec N, Bronowicki JP, Ribard D, Botta-Fridlund D, Hillon P, Besseghir K, Lebrec D. Early administration of vapreotide for variceal bleeding in patients with cir-

rhosis. *N Engl J Med* 2001; **344**: 23-28 [PMID: 11136956 DOI: 10.1056/NEJM200101043440104]
62 **Sung JJ**, Chung SC, Yung MY, Lai CW, Lau JY, Lee YT, Leung VK, Li MK, Li AK. Prospective randomised study of effect of octreotide on rebleeding from oesophageal varices after endoscopic ligation. *Lancet* 1995; **346**: 1666-1669 [PMID: 8551824 DOI: 10.1016/S0140-6736(95)92840-5]
63 **Bañares R**, Albillos A, Rincón D, Alonso S, González M, Ruiz-del-Arbol L, Salcedo M, Molinero LM. Endoscopic treatment versus endoscopic plus pharmacologic treatment for acute variceal bleeding: a meta-analysis. *Hepatology* 2002; **35**: 609-615 [PMID: 11870374 DOI: 10.1053/jhep.2002.31354]
64 **Panés J**, Terés J, Bosch J, Rodés J. Efficacy of balloon tamponade in treatment of bleeding gastric and esophageal varices. Results in 151 consecutive episodes. *Dig Dis Sci* 1988; **33**: 454-459 [PMID: 3280273 DOI: 10.1007/BF01536031]
65 **Monescillo A**, Martínez-Lagares F, Ruiz-del-Arbol L, Sierra A, Guevara C, Jiménez E, Marrero JM, Buceta E, Sánchez J, Castellot A, Peñate M, Cruz A, Peña E. Influence of portal hypertension and its early decompression by TIPS placement on the outcome of variceal bleeding. *Hepatology* 2004; **40**: 793-801 [PMID: 15382120 DOI: 10.1002/hep.20386]
66 **Kalva SP**, Salazar GM, Walker TG. Transjugular intrahepatic portosystemic shunt for acute variceal hemorrhage. *Tech Vasc Interv Radiol* 2009; **12**: 92-101 [PMID: 19853227 DOI: 10.1053/j.tvir.2009.08.003]
67 **Maleux G**, Nevens F, Heye S, Verslype C, Marchal G. The use of carbon dioxide wedged hepatic venography to identify the portal vein: comparison with direct catheter portography with iodinated contrast medium and analysis of predictive factors influencing level of opacification. *J Vasc Interv Radiol* 2006; **17**: 1771-1779 [PMID: 17142707 DOI: 10.1097/01.RVI.0000242185.26944.60]
68 **Lopera JE**. Role of emergency transjugular intrahepatic portosystemic shunts. *Semin Intervent Radiol* 2005; **22**: 253-265 [PMID: 21326703 DOI: 10.1055/s-2005-925551]
69 **Rubin RA**, Haskal ZJ, O'Brien CB, Cope C, Brass CA. Transjugular intrahepatic portosystemic shunting: decreased survival for patients with high APACHE II scores. *Am J Gastroenterol* 1995; **90**: 556-563 [PMID: 7717310]
70 **Sahagun G**, Benner KG, Saxon R, Barton RE, Rabkin J, Keller FS, Rosch J. Outcome of 100 patients after transjugular intrahepatic portosystemic shunt for variceal hemorrhage. *Am J Gastroenterol* 1997; **92**: 1444-1452 [PMID: 9317060]
71 **Bizollon T**, Dumortier J, Jouisse C, Rode A, Henry L, Boillot O, Valette PJ, Ducerf C, Souquet JC, Baulieux J, Paliard P, Trepo C. Transjugular intra-hepatic portosystemic shunt for refractory variceal bleeding. *Eur J Gastroenterol Hepatol* 2001; **13**: 369-375 [PMID: 11338064 DOI: 10.1097/00042737-200104000-00011]
72 **Zizka J**, Eliás P, Krajina A, Michl A, Lojík M, Ryska P, Masková J, Hůlek P, Safka V, Vanásek T, Bukac J. Value of Doppler sonography in revealing transjugular intrahepatic portosystemic shunt malfunction: a 5-year experience in 216 patients. *AJR Am J Roentgenol* 2000; **175**: 141-148 [PMID: 10882264 DOI: 10.2214/ajr.175.1.1750141]
73 **Jirkovsky V**, Fejfar T, Safka V, Hulek P, Krajina A, Chovanec V, Raupach J, Lojik M, Vanasek T, Renc O, Ali SM. Influence of the secondary deployment of expanded polytetrafluoroethylene-covered stent grafts on maintenance of transjugular intrahepatic portosystemic shunt patency. *J Vasc Interv Radiol* 2011; **22**: 55-60 [PMID: 21106389 DOI: 10.1016/j.jvir.2010.09.016]
74 **Sze DY**, Hwang GL, Kao JS, Frisoli JK, Kee ST, Razavi MK, Ahmed A. Bidirectionally adjustable TIPS reduction by parallel stent and stent-graft deployment. *J Vasc Interv Radiol* 2008; **19**: 1653-1658 [PMID: 18823797 DOI: 10.1016/j.jvir.2008.08.011]
75 **Fanelli F**, Salvatori FM, Rabuffi P, Boatta E, Riggio O, Lucatelli P, Passariello R. Management of refractory hepatic encephalopathy after insertion of TIPS: long-term results of shunt reduction with hourglass-shaped balloon-expandable stent-graft. *AJR Am J Roentgenol* 2009; **193**: 1696-1702 [PMID: 19933667 DOI: 10.2214/AJR.09.2968]
76 **Boyer TD**, Haskal ZJ. American Association for the Study of Liver Diseases Practice Guidelines: the role of transjugular intrahepatic portosystemic shunt creation in the management of portal hypertension. *J Vasc Interv Radiol* 2005; **16**: 615-629 [PMID: 15872315 DOI: 10.1097/01.RVI.0000157297.91510.21]
77 **D'Amico G**, Luca A, Morabito A, Miraglia R, D'Amico M. Uncovered transjugular intrahepatic portosystemic shunt for refractory ascites: a meta-analysis. *Gastroenterology* 2005; **129**: 1282-1293 [PMID: 16230081 DOI: 10.1053/j.gastro.2005.07.031]
78 **Bureau C**, Pagan JC, Layrargues GP, Metivier S, Bellot P, Perreault P, Otal P, Abraldes JG, Peron JM, Rousseau H, Bosch J, Vinel JP. Patency of stents covered with polytetrafluoroethylene in patients treated by transjugular intrahepatic portosystemic shunts: long-term results of a randomized multicentre study. *Liver Int* 2007; **27**: 742-747 [PMID: 17617116 DOI: 10.1111/j.1478-3231.2007.01522.x]
79 **Bañares R**, Casado M, Rodríguez-Láiz JM, Camúñez F, Matilla A, Echenagusía A, Simó G, Piqueras B, Clemente G, Cos E. Urgent transjugular intrahepatic portosystemic shunt for control of acute variceal bleeding. *Am J Gastroenterol* 1998; **93**: 75-79 [PMID: 9448179 DOI: 10.1111/j.1572-0241.1998.075_c.x]
80 **Gerbes AL**, Gülberg V, Waggershauser T, Holl J, Reiser M. Transjugular intrahepatic portosystemic shunt (TIPS) for variceal bleeding in portal hypertension: comparison of emergency and elective interventions. *Dig Dis Sci* 1998; **43**: 2463-2469 [PMID: 9824135]
81 **LaBerge JM**, Somberg KA, Lake JR, Gordon RL, Kerlan RK, Ascher NL, Roberts JP, Simor MM, Doherty CA, Hahn J. Two-year outcome following transjugular intrahepatic portosystemic shunt for variceal bleeding: results in 90 patients. *Gastroenterology* 1995; **108**: 1143-1151 [PMID: 7698582 DOI: 10.1016/0016-5085(95)90213-9]
82 **Rajan DK**, Haskal ZJ, Clark TW. Serum bilirubin and early mortality after transjugular intrahepatic portosystemic shunts: results of a multivariate analysis. *J Vasc Interv Radiol* 2002; **13**: 155-161 [PMID: 11830621 DOI: 10.1016/S1051-0443(07)61932-0]
83 **Helton WS**, Belshaw A, Althaus S, Park S, Coldwell D, Johansen K. Critical appraisal of the angiographic portacaval shunt (TIPS). *Am J Surg* 1993; **165**: 566-571 [PMID: 8488938 DOI: 10.1016/S0002-9610(05)80436-2]
84 **McCormick PA**, Dick R, Panagou EB, Chin JK, Greenslade L, McIntyre N, Burroughs AK. Emergency transjugular intrahepatic portasystemic stent shunting as salvage treatment for uncontrolled variceal bleeding. *Br J Surg* 1994; **81**: 1324-1327 [PMID: 7953401 DOI: 10.1002/bjs.1800810922]
85 **Bosch J**. Salvage transjugular intrahepatic portosystemic shunt: is it really life-saving? *J Hepatol* 2001; **35**: 658-660 [PMID: 11690713 DOI: 10.1016/S0168-8278(01)00238-0]
86 **Ferral H**, Patel NH. Selection criteria for patients undergoing transjugular intrahepatic portosystemic shunt procedures: current status. *J Vasc Interv Radiol* 2005; **16**: 449-455 [PMID: 15802443 DOI: 10.1097/01.RVI.0000149508.64029.02]
87 **Owens CA**, Bartolone C, Warner DL, Aizenstein R, Hibblen J, Yaghmai B, Wiley TE, Layden TJ. The inaccuracy of duplex ultrasonography in predicting patency of transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1998; **114**: 975-980 [PMID: 9558286 DOI: 10.1016/S0016-5085(98)70317-8]
88 **Hirooka M**, Ochi H, Koizumi Y, Kisaka Y, Abe M, Ikeda Y, Matsuura B, Hiasa Y, Onji M. Splenic elasticity measured with real-time tissue elastography is a marker of portal hypertension. *Radiology* 2011; **261**: 960-968 [PMID: 21926379

DOI: 10.1148/radiol.11110156]

89 **Gao J**, Ran HT, Ye XP, Zheng YY, Zhang DZ, Wang ZG. The stiffness of the liver and spleen on ARFI Imaging pre and post TIPS placement: a preliminary observation. *Clin Imaging* 2012; **36**: 135-141 [PMID: 22370134 DOI: 10.1016/j.clinimag.2011.11.014]

90 **Harman DJ**, McCorry RB, Jacob RP, Lim TR, O'Neill R, Ryder SD, James MW, Aithal GP, Guha IN. Economic modelling of early transjugular intrahepatic portosystemic shunt insertion for acute variceal haemorrhage. *Eur J Gastroenterol Hepatol* 2013; **25**: 201-207 [PMID: 23089879 DOI: 10.1097/MEG.0b013e32835a4cb0]

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REVIEW

Molecular targeted therapy for hepatocellular carcinoma: Current and future

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Abstract

Hepatocellular carcinoma (HCC) is one of the most frequent tumors worldwide. The majority of HCC cases occur in patients with chronic liver disease. Despite regular surveillance to detect small HCC in these patients, HCC is often diagnosed at an advanced stage. Because HCC is highly resistant to conventional systemic therapies, the prognosis for advanced HCC patients remains poor. The introduction of sorafenib as the standard systemic therapy has unveiled a new direction for future research regarding HCC treatment. However, given the limited efficacy of the drug, a need exists to look beyond sorafenib. Many molecular targeted agents that inhibit different pathways involved in hepatocarcinogenesis are under various phases of clinical development, and novel targets are being assessed in HCC. This review aims to summarize the efforts to target molecular components of the signaling pathways that are responsible for the development and progression of HCC and to discuss perspectives on the future direction of research.

Key words: Hepatocellular carcinoma; Targeted therapy; Molecular agents; Sorafenib

Core tip: Sorafenib is the first drug to prolong survival of patients with advanced hepatocellular carcinoma (HCC). This advance has shifted the paradigm of systemic treatment for HCC toward molecular targeted therapy. This review aims to summarize the efforts to target molecular components of the signaling pathways that are responsible for the development and progression of HCC and to discuss perspectives on the future direction of research.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a common solid cancer and the third most frequent cause of cancer-related mortality worldwide. The 5-year relative survival rate for patients with HCC is only 7%, and very few patients with symptomatic disease survive for > 1 year[1]. One of the primary reasons for the poor prognosis of patients with HCC is the lack of effective treatment options, especially for those with advanced disease. Although surgery and percutaneous ablation can achieve long-term control in some patients with early HCC, fewer than 40% of patients are diagnosed at early stages; hence, only a minority of HCC patients are eligible for potentially curative therapies, such as resection, transplantation, or percutaneous ablation[2]. Furthermore, systemic therapies (such as stan-

dard chemotherapeutic agents) do not provide significant efficacy in HCC based on randomized trials[3].

In recent years, improved knowledge of the oncogenic processes and signaling pathways that regulate tumor cell proliferation, differentiation, angiogenesis, invasion and metastasis has led to the identification of several potential therapeutic targets, which has driven the development of molecularly targeted therapies. An ideal cancer target meets the following criteria: (1) the target is relatively specific for cancer cells (not expressed or expressed at very low levels in normal cells but overexpressed in cancer cells). Meanwhile, overexpression of the target is associated with malignant biological phenotypes and/or poor prognosis; (2) inhibition of the target is efficacious (the target plays an essential role in cancer initiation and progression, and inhibition of expression or activity of the target induces growth suppression and/or apoptosis in cancer cells); and (3) the target is "drugable" as an enzyme (*e.g.*, a kinase) or a cell surface molecule (*e.g.*, a membrane-bound receptor) that can be easily screened for small-molecule inhibitors or targeted by a specific antibody[4].

The aim of this article is to review the efforts to target molecular components of the signaling pathways that are responsible for the development and progression of HCC and to summarize the evidence for the clinical activity of these agents in patients with HCC.

HCC DEVELOPMENT AND SIGNALING PATHWAYS

Hepatocarcinogenesis is a multistep process initiated by external stimuli that lead to genetic changes in hepatocytes or stem cells, resulting in proliferation, apoptosis, dysplasia and neoplasia. The majority of HCC cases are related to chronic viral infections. However, the mechanisms by which hepatitis B virus (HBV) or hepatitis C virus (HCV) induce malignant transformation seem to differ. HBV DNA integrates into the host genome, inducing chromosome instability and insertional mutations that may activate various oncogenes, such as cyclin A[5-7]. Viral proteins, in particular X protein (HBx), act as transactivators to upregulate several oncogenes (such as c-myc and c-jun) and transcriptional factors [(such as nuclear factor-κB][8-10]. Additionally, HBx activates promoters of genes encoding IL-8, tumor necrosis factor (TNF), transforming growth factor (TGF)-β and epidermal growth factor receptor (EGFR)[11]. HBx can also stimulate several signal transduction pathways, including the JAK/STAT, RAS/RAF/MAPK, and Wnt/β-catenin pathways[12-14].

The contributions of HCV to hepatocarcinogenesis are mediated by viral proteins, including core, NS3 and NS5A proteins. HCV core protein can promote apoptosis or cell proliferation through interaction with p53 or upregulation of Wnt-1 at the transcriptional level[15,16]. NS4A and NS4B proteins mediate translational inhibition and degradation of various cellular proteins[17]. Cirrhosis is present in approximately 80%-90% of HCC patients and constitutes the largest single risk factor. In cirrhotic liver, changes in fat metabolism associated with the activation of adipocyte-like pathways are thought to be involved in neoplastic transformation[18].

MAPK PATHWAY (RAS/RAF/MEK/ERK)

The Raf/MAPK/extracellular-signal-regulated kinase (ERK) pathway is an important pro-survival signaling pathway that is primarily involved in cell growth and survival and regulates cell differentiation. This pathway transduces extracellular signals form membrane-bound tyrosine kinase receptors, such as EGFR, insulin-like growth factor receptor (IGFR), vascular endothelial growth factor receptor (VEGFR), c-Met and platelet-derived growth factor receptor (PDGFR), to the nucleus. Growth factor binding results in receptor phosphorylation, which activates an adapter molecule complex known as GRB2/SHC/SOS. This sequence in turn activates the RAF/mitogen/extracellular protein kinase (MEK)/ERK pathway, which triggers a cascade of specific phosphorylation events[19]. Within this pathway, the small GTPase RAS and the serine/threonine kinase Raf are the key signal regulators[20]. Intermediate signaling is regulated by MEK1 and MEK2, which are responsible for phosphorylating and activating the final downstream signaling molecules extracellular-regulated protein kinases (ERK)1 and ERK2[21]. ERK1/2 regulates cellular activity by acting on more than 100 substrates in the cytoplasm and nucleus. RAS also regulates the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, the phospholipase C/protein kinase C pathway and the ral guanine nucleotide dissociation stimulator pathway[22,23].

Up-regulated activation of the Raf/MAPK/ERK signaling pathway has been well documented in HCC and correlates with advanced stage[24,25]. Mechanisms for the increased activity of the Raf/MAPK/ERK signaling pathway in HCC include down-regulation of Raf kinase inhibitor protein (a suppressor of the Raf/MAPK/ERK pathway) and induction by hepatitis viral proteins (such as the hepatitis B X protein and the hepatitis C core protein)[26-28].

Targeting Raf kinase is one of the most promising targeted approaches for the treatment of HCC. Sorafenib has strong inhibitory activity against Raf-1 (C-Raf) kinase and B-Raf (wild-type B-Raf and mutant V600E B-Raf) and has been shown to inhibit other serine/threonine kinases, the pro-angiogenic receptor tyrosine kinases VEGFR, PDGFR and FGFR1, and tyrosine kinases such as c-kit, Flt-3 and RET, which are involved in tumor progression and overall prognosis (Figure 1)[29].

Selumetinib (AZD6244) is an oral non-ATP-competitive small-molecule inhibitor of the mitogen-activated protein kinase MEK1/2. A recent study has shown that selumetinib plus rapamycin exerts antitumor and antiangiogenic effects in preclinical models of human HCC[30]. In a phase Ⅰ/Ⅱ study of selumetinib in combination with

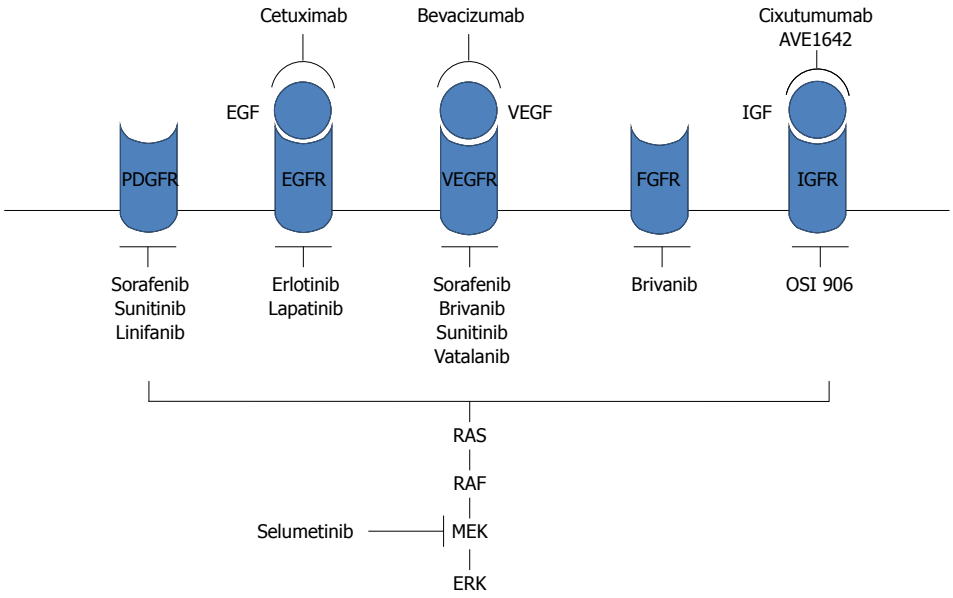

Figure 1 Ras/Raf/MEK/ERK signaling pathways and molecular targeted agents which is currently available or in development for hepatocellular carcinoma. EGF: Epidermal growth factor; EGFR: EGF receptor; ERK: Extracellular signal-regulated kinase; FGFR: Fibroblast growth factor receptor; IGFR: Insulin-like growth factor receptor; PDGFR: Platelet-derived growth factor receptor; VEGF: Vascular endothelial growth factor; VEGFR: VEGF receptor; MEK: Mitogen/extracellular protein kinase.

sorafenib in advanced HCC, the objective responses were 3 partial response (PR), 6 stable disease (SD) and 2 progressive disease (PD) among 11 patients, and the common toxicities were diarrhea, rash, fatigue, and hypertension[31].

Another phase Ⅰ/Ⅱ study has evaluated the combination of the MEK inhibitor RDEA119 and sorafenib in patients with advanced cancer (NCT00785226).

PI3K/AKT/MTOR PATHWAY

The PI3K/Akt/mTOR pathway also plays an important role in cell growth, survival regulation, metabolism and anti-apoptosis[32]. The binding of growth factors (such as IGF and EGF) to their receptors activates PI3K[19]. PI3K subsequently produces the lipid second messenger PIP3b (phospho-inositol triphosphate), which in turn activates the serine/threonine kinase AKT. Activated AKT also phosphorylates several cytoplasmic proteins, most notably mTOR and BCL-2-associated death promoter[19]. The activation of mTOR increases cellular proliferation, and inactivation of BAD decreases apoptosis and increases cell survival[21]. In normal tissue, this pathway is negatively regulated by the tumor suppressor phosphatase on chromosome 10 [phosphatase and tensin homolog (PTEN)], which targets the lipid products of PI3K for dephosphorylation[21].

Expression of both IGF and the IGF receptor is up-regulated in HCC and cirrhotic liver, resulting in stimulation of the PI3K/AKT/mTOR signaling pathway in addition to activation of the RAF/MEK/ERK and WNT/β-catenin pathways[33,34]. Anomalies in PTEN function may lead to overactivation of the PI3K/AKT/mTOR pathway in HCC. PTEN expression is reduced in nearly half of all HCC tumors, resulting in constitutive activation of the PI3K/AKT/mTOR pathway[35]. Decreased PTEN expression has been shown to correlate with increased tumor grade, advanced disease stage and reduced overall survival (OS) in patients with HCC[35]. In a mutation analysis of HCC samples, activation of the IGF pathway, upregulation of EGF, dysregulation of PTEN, and aberrant mTOR signaling were present in half of the samples; inhibition of mTOR activity with a rapamycin analog (everolimus) was effective in improving survival and suppressing recurrence[36]. These results suggest that mTOR pathway activation plays a crucial role in the pathogenesis of HCC (Figure 2).

The PI3K inhibitor RG7321 and the Akt inhibitor perifosine target the PI3K/Akt/mTOR pathway and are in early stages of clinical development. The mTOR inhibitors everolimus (RAD001), sirolimus (Rapamune) and temsirolimus (CCI-779) have been studied for efficacy and safety in patients with advanced HCC. Everolimus has produced a median progression-free survival (PFS) of 3.8 mo and OS of 8.4 mo in phase Ⅰ/Ⅱ testing in patients with advanced HCC[37]. A phase Ⅲ study to compare everolimus and placebo and a phase Ⅰ/randomized phase Ⅱ study (sorafenib + everolimus *vs* sorafenib alone) to test the efficacy and tolerance of sorafenib in combination with everolimus are underway (NCT01035229). In a phase Ⅱ study of sirolimus in patients with advanced HCC, sirolimus exhibited some antitumor activity in patients with advanced HCC[38]. However, larger studies are required to determine the value of this agent.

Temsirolimus, an mTOR inhibitor, has been approved for the treatment of advanced renal cell carcinoma. The efficacy and potential utility of this agent in HCC is currently under investigation (NCT01079767).

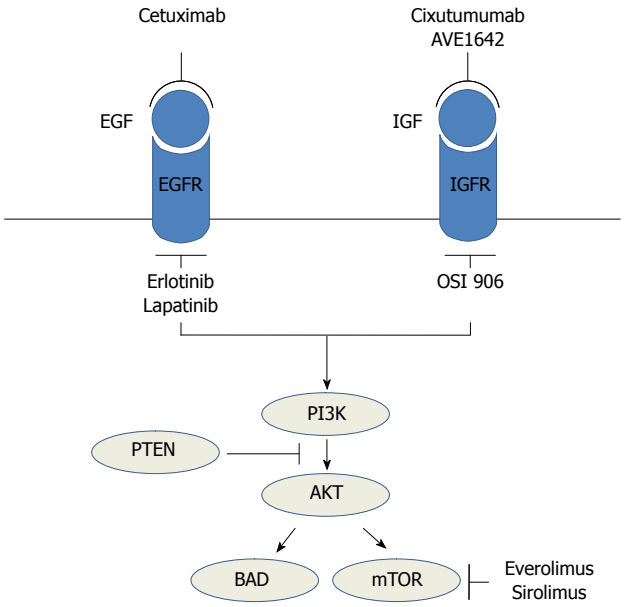

Figure 2 PI3K/Akt/mTOR pathway and the molecular agents targeting this pathway. BAD: BCL-2-associated death promoter; EGF: Epidermal growth factor; EGFR: EGF receptor; IGFR: Insulin-like growth factor (IGF) receptor; mTOR: Mammalian target of rapamycin; PTEN: Phosphatase and tensin homolog; PI3K: Phosphatidylinositol-3-kinase.

VEGF/VEGFR, PDGFR, AND FGFR

Normal angiogenesis is maintained by a balance between proangiogenic and anti-angiogenic factors[39]. The angiogenic balance is disturbed in HCC. Angiogenesis is important for HCC growth and metastasis and occurs as a result of complex alterations that involve promoting factors [such as VEGF, angiopoietin and fibroblast growth factor (FGF), inhibitory factors, including thrombospondin (TSP) and angiostatin], and the surrounding tissue. A number of angiogenic growth factors, including VEGF-A, angiopoietin-2 and PDGF, have been shown to be upregulated in HCC tumors at the gene expression level and plasma protein level in patients with HCC compared with cirrhotic patients[40]. The principal angiogenic factors involved are VEGFs, PDGFs, TGF-alpha and -beta, basic FGF, EGF, hepatocyte growth factor (HGF), angiopoietins and interleukin-4 and -8[39,41]. These growth factors and cytokines induce angiogenic signaling through a variety of mechanisms, including activation of the RAF/MEK/ERK, PI3K/AKT/mTOR and JAK/signal transducer and activator pathways.

Increased VEGF expression has been reported in cirrhotic and dysplastic liver tissue, suggesting a possible role for VEGF-mediated angiogenesis in hepatocarcinogenesis[42]. VEGF clearly plays an important regulatory role in HCC; high levels of VEGF expression have been linked with HCC tumor grade, poor outcome after resection, disease recurrence, poor disease-free survival (DFS) and OS, vascular invasion and portal vein emboli[43-46]. Expression of FGF-2 is also elevated in patients with HCC, and FGF-2 expression in HCC correlates with tumor microvessel density and postoperative recurrence rate[47-49]. Tumor angiogenin expression correlates with microvascular density in patients with HCC, and high serum angiogenin levels are associated with decreased survival at 5 years[50].

The VEGF pathway can be targeted through two approaches: anti-VEGF monoclonal antibodies or inhibitors of the receptor tyrosine kinase associated with VEGFR. The anti-VEGF monoclonal antibody bevacizumab was the first angiogenesis inhibitor to be approved as an antineoplastic agent. Bevacizumab has shown encouraging early evidence of efficacy in patients with advanced HCC[51,52]. The combination of bevacizumab with either cytotoxic agents (gemcitabine, oxaliplatin, and capecitabine) or erlotinib has also shown encouraging results in four phase Ⅱ trials in patients with advanced HCC[53-56].

Sorafenib is an orally available multikinase inhibitor that was originally designed to target VEGFR-1, -2, -3, PDGFR and c-kit. In a phase Ⅱ study of patients with advanced inoperable HCC, sorafenib induced a PR in 2% of the patients. The median time to progression (TTP) was 4.2 mo and median OS was 9.2 mo[57]. In the phase Ⅲ SHARP (Sorafenib HCC Assessment Randomized Protocol) trial, sorafenib (400 mg twice daily) significantly prolonged OS compared with placebo in patients with advanced HCC (10.7 mo in the sorafenib group *vs* 7.9 mo in the placebo group)[58]. The median time to radiological progression was significantly longer in the sorafenib group (5.5 mo *vs* 2.8 mo). In another randomized phase Ⅲ study performed in the Asia-Pacific region, the OS was 6.5 mo in the sorafenib group compared with 4.2 mo in the placebo group (the hazard ratio in the sorafenib group was 0.68, $P = 0.014$)[59]. Sorafenib is the only targeted therapy to have been approved for clinical use in several countries, including the United States and in Europe. Although sorafenib improved OS in patients with HCC, the associated toxicities may significantly affect patients' quality of life. High rates of dermatologic side effects have commonly been reported with sorafenib, the most clinically significant of which is hand-foot skin reaction[60]. Despite initial responses to sorafenib, most HCC patients experience a loss of efficacy. No effective second-line treatment options currently exist for patients who are resistant or refractory to and/or intolerant of sorafenib.

Beyond sorafenib, sunitinib is the most studied multikinase inhibitor targeting VEGFR-1 and VEGFR-2. Sunitinib also displays inhibitory activities against other receptor tyrosine kinases, including PDGFR-a/b, c-KIT, FLT3, and RET kinases. Sunitinib is currently indicated for the treatment of renal cell carcinoma and gastrointestinal stromal tumors[61-63]. Two phase Ⅱ studies of sunitinib in patients with advanced HCC have been performed. In the first study, the PR rate was 2.9%, and 50% of the patients achieved stable disease[64]. In a second phase Ⅱ study, one (2.7%) patient experienced a confirmed PR and 13 (35%) of 37 patients achieved stable

disease[65]. A phase Ⅲ study comparing sunitinib with sorafenib (NCT00699374) was discontinued due to a greater incidence of adverse events in the sunitinib group and because sunitinib failed to demonstrate superiority or non-inferiority to sorafenib in extending the survival of patients with advanced HCC.

Brivanib is a dual inhibitor of VEGFR and fibroblast growth factor receptor signaling pathways. Because FGF signaling may contribute to acquired "resistance" or compensatory signaling during anti-VEGFR therapy, the simultaneous inhibition of these 2 pathways by brivanib may both delay initial progression in response to antiangiogenic therapy (as first-line treatment) and successfully treat tumors that have already progressed during the course of anti-VEGFR therapy (as second-line treatment)[66,67]. Brivanib has demonstrated a disease control rate of 51% and an OS of 10 mo as first-line monotherapy in a phase Ⅱ trial of predominantly Asian patients with HCC[68]. In another phase Ⅱ trial of brivanib in patients with HCC who had been treated with sorafenib, brivanib caused a tumor response rate of 4.3% and disease control rate of 45.7%[69].

Large randomized phase Ⅲ Brivanib Study in Patients at Risk (BRISK) HCC program trials have been conducted to evaluate the role of brivanib in advanced HCC (BRISK-FL, BRISK-PS and BRISK-APS). The BRISK-PS trial evaluated brivanib *vs* placebo in patients who had failed or were intolerant to sorafenib therapy (NCT00825955). This study did not meet its primary end point of improving OS, but treatment with brivanib showed improvements in the response rate[70]. The BRISK-FL trial (NCT00858871) directly compared the clinical outcomes of brivanib *vs* sorafenib in patients with advanced HCC who received no prior systemic therapy. The median OS was 9.5 mo in the brivanib arm compared with 9.9 mo in the sorafenib arm, which was not a statistically significant difference. No significant survival differences were observed between subgroups based on geographic regions, cause of HCC or disease severity. The study did not meet its primary OS objective based upon a non-inferiority statistical design[71].

Vatalanib (PTK787) is a potent tyrosine kinase inhibitor that binds directly to the ATP-binding sites of VEGF receptors. Vatalanib inhibits both Flt-1 and Flk-1/KDR and other class Ⅲ receptor tyrosine kinases, such as PDGFR-β, Flt-4, c-kit, and c-fms[72]. In a phase Ⅰ/Ⅱ study of vatalanib combined with doxorubicin in patients with advanced HCC, the overall response rate was 26.0%, with all of the responding patients achieving PR. Another 20% of the patients achieved SD for at least 12 wk[73].

Linifanib (ABT-869) is a novel receptor tyrosine kinase inhibitor with potent activity against members of the VEGFR and PDGFR families[74]. In a phase Ⅱ study of linifanib in advanced HCC, the estimated objective response rate was 9.1%, the median time to disease progression was 3.7 mo, and the median OS was 9.7 mo[75]. An open-label, randomized phase Ⅲ study of the efficacy and tolerability of linifanib *vs* sorafenib in advanced HCC (NCT01009593) was conducted. The OS of linifanib given as monotherapy once daily was similar to sorafenib given twice daily per standard of care[76].

TSU-68 is an oral tyrosine kinase inhibitor of FGFRs, VEGFRs and PDGFR and has demonstrated some clinical efficacy in a phase Ⅰ/Ⅱ trial of heavily pretreated patients with advanced HCC. Treatment of patients with unresectable or metastatic HCC with TSU-68 was associated with disease stabilization or improvement in 51% of the patients[77]. A randomized placebo-controlled phase Ⅲ trial in Japan, South Korea and Taiwan is currently recruiting patients with unresectable HCC and will evaluate transcatheter arterial chemoembolization (TACE) in combination with either TSU-68 or placebo.

Cediranib (AZD2171) is another selective inhibitor of VEGFR-1, -2 and -3. Cediranib also exhibits activity against c-kit, PDGFR-β, and FLT4 at nanomolar concentrations. In a phase Ⅱ clinical study of advanced HCC, the median OS was 5.8 mo. No patients experienced confirmed response. The median time to progression was 2.8 mo[78].

EGFR, IGF AND HGF/c-MET SIGNALING

EGFR, a member of the human epidermal growth factor receptor (HER) family, contains an intracellular tyrosine kinase domain which can trigger signal transduction through the MAPK and PI3K/Akt/mTOR pathways. Thus, these receptors contribute to cell growth, differentiation, survival and adhesion[79]. EGFR overexpression has been reported in HCC. Immunohistochemical analysis by Buckley *et al*[80] revealed that EGFR was overexpressed in 50 (66%) of 76 HCCs, and fluorescence *in situ* hybridization (FISH) showed additional *EGFR* gene copies in 17 (45%) of 38 HCCs. EGFR-targeting drugs include anti-EGFR antibodies (such as cetuximab and panitumumab) and inhibitors of EGFR tyrosine kinases (such as erlotinib, lapatinib and gefitinib); these drugs have been used widely for the treatment of HCC.

Cetuximab is a recombinant chimeric monoclonal antibody that targets the extracellular domain of EGFR. In a phase Ⅱ clinical trial of cetuximab in patients with advanced HCC, the median OS was 9.6 mo and the median PFS was 1.4 mo. The treatment was generally well tolerated. No treatment-related grade 4-5 toxicities occurred. Grade 3 aspartate aminotransferase, hypomagnesemia, and fever without neutropenia were each noted in 1 patient[81]. A randomized trial comparing gemcitabine-oxaliplatin (GEMOX) alone with a GEMOX-cetuximab combination is ongoing to define the real contribution of anti-EGFR therapy.

Erlotinib is a potent and reversible inhibitor of EGFR tyrosine kinase. In an *in vitro* study, erlotinib potently suppressed the growth of human EGFR-expressing HCC cell lines. Erlotinib has been shown to inhibit the RAF/MEK/ERK signaling pathway and block signal

transducer and activator of transcription-mediated signaling[82]. A phase Ⅲ placebo-controlled, double-blind SEARCH (Sorafenib and Erlotinib, a Randomized Trial Protocol for the Treatment of Patients with HCC) trial has been conducted in patients with advanced HCC. Three hundred sixty-two patients received sorafenib plus erlotinib and 358 received sorafenib plus placebo. No significant differences were observed in OS or TTP between the arms. Erlotinib, when added to sorafenib as the standard of care in advanced HCC, did not prolong overall survival[83].

Lapatinib is a dual inhibitor of EGFR and HER-2/NEU that acts by docking into the ATP binding site of the two receptors[84]. Phase Ⅱ results have indicated that lapatinib is well tolerated and have shown preliminary evidence of antitumor activity in HCC[85]. Among 40 patients with advanced HCC, the response rate was 5%, median PFS was 2.3 mo and median OS was 6.2 mo.

The IGF/IGFR signaling pathway regulates several cellular processes, including proliferation, motility and inhibition of apoptosis[86]. Ligand binding to IGF-1R triggers rapid receptor autophosphorylation, which in turn initiates downstream cellular effectors, ultimately leading to activation of PI3K, protein kinase B and the RAF/MEK/ERK pathway[87]. In HCC, dysregulation of IGF signaling occurs predominantly at the level of IGF-2. IGF-2 is overexpressed in 16%-40% of human HCCs, and IGF-2R (an alternative receptor for IGF-2) is underexpressed in approximately 80% of HCCs[88,89]. Associations have been reported between disease stage, metastasis and survival and the functions of IGF and IGFR in HCC[90,91]. Several strategies to target this system, including monoclonal antibodies against the IGF-1 receptor (IGF-1R) and small molecule inhibitors of the tyrosine kinase function of IGF-1R, are under active investigation.

Pre-clinical evidence obtained from HCC cells has shown that IMC-A12 (cituxumumab), a human monoclonal antibody that blocks IGF-1R. A phase Ⅰ study of IMC-A12 yielded a partial response in HCC[92]. However, a subsequent phase Ⅱ study in patients with advanced HCC showed that IMC-A12 is inactive as a monotherapy[93]. Up to 46% of the patients developed grade 3-4 hyperglycemia in this study. Hyperglycemia may be the dose limiting toxicity of IGF-1R monoclonal antibodies.

BIIB022 is an anti-IGF-1R monoclonal antibody that blocks binding of both IGF-1 and IGF-2 to IGF-1R. This agent does not appear to cause hyperglycemia, which is a common side effect of receptor-specific antibodies. A planned phase Ⅰ/Ⅱ study comparing sorafenib with or without BIIB022 in patients with advanced HCC was terminated due to a business decision by the sponsor company.

AVE1642 is another monoclonal antibody that specifically blocks IGF-1R signaling. This agent has been evaluated in combination with sorafenib in a phase Ⅰ study in advanced HCC patients[94]. Long-lasting disease stabilization was observed in most patients with PD.

OSI-906 is a novel potent dual tyrosine kinase inhibitor of both IGF-1R and insulin receptor. The unique advantage of OSI-906 over the previous class of anti-IGF drugs is its ability to minimize IGF-2 activity in situations in which IGF-1R inhibition alone is not sufficient. The phase Ⅱ study of second-line treatment for advanced HCC patients who failed first-line treatment with sorafenib (NCT01101906) was terminated because the sponsor decided not to pursue the development of this drug.

The HGF/Met pathway is involved in tumor growth, invasion and angiogenesis in various types of cancer[95]. c-Met is a tyrosine kinase receptor for the HGF ligand. HGF-induced activation of c-MET ultimately leads to the activation of downstream effecter molecules, including phospholipase C, PI3K and ERK[96]. c-MET overexpression has been observed in 20%-48% of HCC, and overexpression has been linked with decreased 5-year survival in patients with HCC (FIgure 3)[97-99].

Tivantinib (ARQ 197) is a selective, oral MET receptor tyrosine kinase inhibitor with broad-spectrum antitumor activity as single agent. MET overexpression has been shown to be a negative prognostic factor in HCC after sorafenib failure. Tivantinib demonstrated a nearly doubling of PFS and OS in the MET high group compared to placebo in a phase Ⅱ study as second-line treatment in patients with advanced HCC[100]. The activity of tivantinib in combination with sorafenib is also promising. Adverse events include hematological toxicity, asthenia and loss of appetite. The initially high incidence of neutropenia in patients with HCC led to dose reduction from 360 mg *bid* to 240 mg *bid*. Currently, a pivotal phase Ⅲ study in advanced, MET-high HCC after sorafenib failure is planned.

WNT-BETA-CATENIN PATHWAY

A major and early carcinogenic event in the development of HCC seems to be the abnormal regulation of the transcription factor β-catenin, a key component of the WNT signaling pathway.

During normal cell homeostasis, Wnt proteins are absent. Initiation of Wnt signaling leads to a series of events that cause loss of β-catenin phosphorylation, which prevents its degradation. β-catenin then accumulates in the cytoplasm and translocates into the nucleus. Hepatocytes with nuclear translocation of β-catenin display abnormal cellular proliferation and express membrane proteins involved in HCC, metastatic behavior, and cancer stem cells[101]. A high incidence of β-catenin mutations (nearly 40%) has been observed in HCC cases that occur in patients with HCV. HCC cases that occur in HBV patients display β-catenin activation that is induced in a mutation-dependent manner by the expression of HBx protein[102,103]. Agents targeting Wnt-β-catenin are under development. Preliminary studies targeting the Wnt-β-catenin pathway have demonstrated a potential space for new novel therapies to treat HCC.

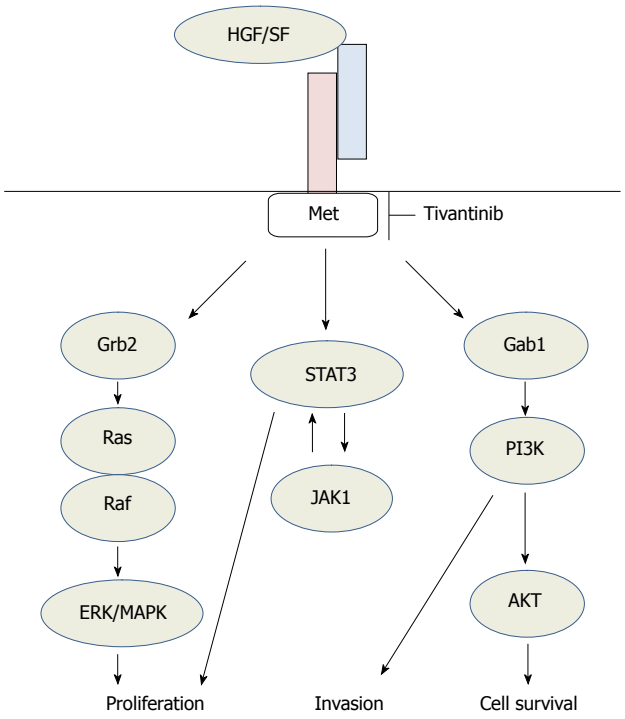

Figure 3 The c-Met signaling pathway suggested in hepatocellular carcinoma. Gab1: GRB2-associated binding protein 1; Grb2: Growth factor receptor-bound protein 2; HGF/SF: Hepatocyte growth factor/scatter factor; JAK1: Janus kinase 1; Met: Met proto-oncogene; PI3K: Phosphatidylinositol-3-kinase; STAT3: Signal transducer and activator of transcription 3; ERK: Extracellular signal-regulated kinase.

Table 1 Molecular targets and therapeutic agents

Molecular targets	Therapeutic agents
VEGF/VEGFR	Sorafenib
	Bevacizumab
	Vatalanib (PTK787)
	Cediranib (AZD2171)
	Brivanib
	Sunitinib
	Linifanib (ABT869)
EGF/EGFR	Cetuximab
	Erlotinib
	Lapatinib
IGF/IGFR	OSI-906
	IMC-A12
	AVE1642
	BIIB022
Ras/Raf/MEK/ERK	Sorafenib
	Selumetinib (AZD6244)
PI3K/Akt/mTOR	AZD8055
	Everolimus
	Sirolimus
	Temsirolimus
Wnt-β-catenin	PFK118-310
	PFK115-584
	CGP049090
MET	Tivanitib

EGF: Epidermal growth factor; EGFR: EGF receptor; ERK: Extracellular signal-regulated kinase; FGFR: Fibroblast growth factor receptor; IGFR: Insulin-like growth factor (IGF) receptor; VEGF: Vascular endothelial growth factor; VEGFR: VEGF receptor; mTOR: Mammalian target of rapamycin; MET: Met proto-oncogene.

JAK/STAT PATHWAY

The Jak/Stat pathway is activated by more than 40 cytokines and growth factors and is involved in multiple cell functions, including differentiation, proliferation, and apoptosis[104]. In this pathway, cytokines induce phosphorylation of the Janus tyrosine kinases (Jak1, 2 and 3 and Tyk2), which is followed by activation of Stat1-6[105]. The phosphorylation of Jak1, Jak2, and Tyk2 tyrosine kinases is not detected in normal livers but increases significantly between surrounding non-neoplastic liver and HCCs[106]. Activation of Stat1, Stat3, and Stat5 has been shown to be significantly higher in tumors than in the respective surrounding livers; pStat3 is higher in HCC with poor prognosis than in HCC with better prognosis[106]. The levels of Jak/Stat targets, including Bcl-xl, Mcl-1, cyclin D1, and c-Myc, are markedly elevated in the majority of HCCs. A phase I study of the JAK2 inhibitor AZD1480 in advanced solid malignancy (including HCC) is planned (NCT01219543).

FUTURE PERSPECTIVES

Molecular targeted agents that have been introduced into clinical use in recent years have been approved for the treatment of a specific cancer and then frequently used to treat various other types of cancer (Table 1). Genetic alterations clearly play a major role in hepatocarcinogenesis, and abnormalities in several critical molecular signaling pathways have been identified as contributing to tumor development and progression[107,108].

Currently, sorafenib is the only effective systemic treatment option for advanced HCC. While the drug is effective for patients with advanced HCC, sorafenib prolongs life expectancy for only approximately three mo. To move beyond sorafenib monotherapy, a potential role for this agent in the adjuvant setting following surgical resection, radiofrequency ablation, or TACE or in combination with other targeted agents or chemotherapy is under investigation.

Several new promising multi-targeted molecules have been developed and are currently under investigation for the treatment of HCC (Table 2). Unfortunately, HCCs are refractory to many targeted therapies. Therefore, resistance to treatment remains the major challenge for targeted therapy. Many resistance mechanisms have been identified, including epigenetic changes, alternative splicing, target inactivation, upregulation of alternative pathways (by cellular adaptation to the pathway being targeted), and a range of mutations. A combination of different agents or a single "unspecific" inhibitor of several pathways may offer advantages to overcome resistance. Combinations of targeted agents with chemotherapy regimens also remain to be further explored. Molecular targeted therapy blocking angiogenesis has demonstrated somewhat promising results, but the efficacy of these agents is limited by survival pathways induced by hypoxia. Thus, the inhibition of hypoxia-induced survival signals

Table 2 Efficacy results of targeted therapies for advanced hepatocellular carcinoma

Molecular targets/agents	Phase	Efficacy	Ref.
VEGF/VEGFR			
Sorafenib	Phase Ⅲ SHARP Sorafenib *vs* placebo	Median OS: 10.7 mo *vs* 7.9 mo	[58]
	Phase Ⅲ (Asian)	Median OS:6.5 mo *vs* 4.2 mo	[59]
Sunitinib	Phase Ⅱ	Median PFS: 3.9 mo Median OS: 9.8 mo	[65]
	Phase Ⅲ Sunitinib *vs* sorafenib	Median OS: 7.9 mo *vs* 10.2 mo	
Brivanib	Phase Ⅱ, first-line	Median PFS: 2.8 mo Median OS: 10 mo	[68]
	Phase Ⅱ, second-line	Median PFS: 2.7 mo Median OS: 9.8 mo	[69]
	Phase Ⅲ (BRISK-PS) Brivanib *vs* placebo	Median OS: 9.4 mo *vs* 8.3 mo TTP: 4.2 mo *vs* 2.7 mo RR: 12% *vs* 2%	[70]
	Phase Ⅲ (BRISK-FL) Brivanib *vs* placebo	Median OS: 9.5 mo *vs* 9.9 mo TTP: 4.2 mo *vs* 4.1 mo RR: 12% *vs* 8%	[71]
Vatalanib (PTK787)	Phase Ⅰ/Ⅱ, combined with doxorubicin	OS: 7.3 mo PFS: 5. 4 mo	[73]
Inifanib (ABT-869)	Phase Ⅱ	TTP: 3.7 mo Median OS: 9.7 mo	[75]
Cediranib (AZD2171)	Phase Ⅱ	Median OS: 5.8 mo TTP: 2.8 mo	[78]
EGF/EGFR			
Cetuximab	Phase Ⅱ	Median OS: 9.6 mo Median PFS: 1.4 mo	[81]
Erlotinib	Phase Ⅲ (SEARCH) Sorafenib/erlotinib *vs* orafenib/placebo	Median OS: 9.5 mo *vs* 8.5 mo TTP: 3.2 mo *vs* 4.0 mo	[83]
Lapatinib	Phase Ⅱ	Median PFS: 2.3 mo Median OS: 6.2 mo	[85]
	Phase Ⅲ Lipatinib *vs* sorafenib	Median OS: 9.1 mo *vs* 9.8 mo	
IGF/IGFR			
Cituxumumab (IMC-A12)	Phase Ⅱ	Median OS: 8 mo	[93]
Ras/Raf/MEK/ERK			
Selumetinib (AZD6244)	Phase Ⅰ/Ⅱ	11 patients enrolled PR in 3, SD in 6, PD in 2 patients	[31]
PI3K/Akt/mTOR			
Everolimus	Phase Ⅰ/Ⅱ	Median PFS: 3.8 mo Median OS: 8.4 mo	[37]
Sirolimus	Phase Ⅱ	Median PFS: 15.3 wk Median OS: 26.4 wk	[38]
MET			
Tivantinib	Randomized Phase Ⅱ Tivantinib *vs* placebo ITT population	Median TTP: 6.9 wk *vs* 6.0 wk Median OS: 6.6 mo *vs* 6.2 wk	[100]
	c-Met high	Median TTP: 11.7 wk *vs* 6.1 wk Median OS: 7.2 mo *vs* 3.8 wk	

ITT: Intent to treat; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival; PR: Partial response; RR: Response rate; SD: Stable disease; TTP: Time to progression; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; mTOR: Mammalian target of rapamycin; PI3K: Phosphatidylinositol-3-kinase; Met: Met proto-oncogene; EGFR: Epidermal growth factor (EGF) receptor.

might be required for targeted agents to block angiogenesis as an adjuvant therapy following TACE. Additionally, exploring potential markers that can help in identifying the patients who are most likely to respond (or to at least identify those who will not respond) to treatment is critical. Future development of genomic analysis of HCC will aid in the identification of specific biomarkers for patient selection for either single agent or combination molecular targeted therapies.

REFERENCES

1 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16 [PMID: 15508102 DOI: 10.1053/j.gastro.2004.09.011]

2 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/S0140-6736(03)14964-1]

3 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID:

12540794 DOI: 10.1053/jhep.2003.50047]
4 **Sarker D**, Molife R, Evans TR, Hardie M, Marriott C, Butzberger-Zimmerli P, Morrison R, Fox JA, Heise C, Louie S, Aziz N, Garzon F, Michelson G, Judson IR, Jadayel D, Braendle E, de Bono JS. A phase I pharmacokinetic and pharmacodynamic study of TKI258, an oral, multitargeted receptor tyrosine kinase inhibitor in patients with advanced solid tumors. *Clin Cancer Res* 2008; **14**: 2075-2081 [PMID: 18381947 DOI: 10.1158/1078-0432.CCR-07-1466]
5 **Brechot C**, Pourcel C, Louise A, Rain B, Tiollais P. Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature* 1980; **286**: 533-535 [PMID: 6250074 DOI: 10.1038/286533a0]
6 **Minami M**, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, Okanoue T. Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. *Oncogene* 2005; **24**: 4340-4348 [PMID: 15806150 DOI: 10.1038/sj.onc.1208628]
7 **Wang J**, Chenivesse X, Henglein B, Bréchot C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature* 1990; **343**: 555-557 [PMID: 1967822 DOI: 10.1038/343555a0]
8 **Balsano C**, Avantaggiati ML, Natoli G, De Marzio E, Will H, Perricaudet M, Levrero M. Full-length and truncated versions of the hepatitis B virus (HBV) X protein (pX) transactivate the cmyc protooncogene at the transcriptional level. *Biochem Biophys Res Commun* 1991; **176**: 985-992 [PMID: 1645550 DOI: 10.1016/0006-291X(91)90379-L]
9 **Twu JS**, Lai MY, Chen DS, Robinson WS. Activation of protooncogene c-jun by the X protein of hepatitis B virus. *Virology* 1993; **192**: 346-350 [PMID: 8390762 DOI: 10.1006/viro.1993.1041]
10 **Chirillo P**, Falco M, Puri PL, Artini M, Balsano C, Levrero M, Natoli G. Hepatitis B virus pX activates NF-kappa B-dependent transcription through a Raf-independent pathway. *J Virol* 1996; **70**: 641-646 [PMID: 8523586]
11 **Andrisani OM**, Barnabas S. The transcriptional function of the hepatitis B virus X protein and its role in hepatocarcinogenesis (Review). *Int J Oncol* 1999; **15**: 373-379 [PMID: 10402250]
12 **Lee YH**, Yun Y. HBx protein of hepatitis B virus activates Jak1-STAT signaling. *J Biol Chem* 1998; **273**: 25510-25515 [PMID: 9738022 DOI: 10.1074/jbc.273.39.25510]
13 **Benn J**, Schneider RJ. Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. *Proc Natl Acad Sci USA* 1994; **91**: 10350-10354 [PMID: 7937954 DOI: 10.1073/pnas.91.22.10350]
14 **Cha MY**, Kim CM, Park YM, Ryu WS. Hepatitis B virus X protein is essential for the activation of Wnt/beta-catenin signaling in hepatoma cells. *Hepatology* 2004; **39**: 1683-1693 [PMID: 15185310 DOI: 10.1002/hep.20245]
15 **Yamanaka T**, Kodama T, Doi T. Subcellular localization of HCV core protein regulates its ability for p53 activation and p21 suppression. *Biochem Biophys Res Commun* 2002; **294**: 528-534 [PMID: 12056798 DOI: 10.1016/S0006-291X(02)00508-9]
16 **Fukutomi T**, Zhou Y, Kawai S, Eguchi H, Wands JR, Li J. Hepatitis C virus core protein stimulates hepatocyte growth: correlation with upregulation of wnt-1 expression. *Hepatology* 2005; **41**: 1096-1105 [PMID: 15841445 DOI: 10.1002/hep.20668]
17 **Florese RH**, Nagano-Fujii M, Iwanaga Y, Hidajat R, Hotta H. Inhibition of protein synthesis by the nonstructural proteins NS4A and NS4B of hepatitis C virus. *Virus Res* 2002; **90**: 119-131 [PMID: 12457968 DOI: 10.1016/S0168-1702(02)00146-6]
18 **Terasaki S**, Kaneko S, Kobayashi K, Nonomura A, Nakanuma Y. Histological features predicting malignant transformation of nonmalignant hepatocellular nodules: a prospective study. *Gastroenterology* 1998; **115**: 1216-1222 [PMID: 9797377 DOI: 10.1016/S0016-5085(98)70093-9]
19 **Avila MA**, Berasain C, Sangro B, Prieto J. New therapies for hepatocellular carcinoma. *Oncogene* 2006; **25**: 3866-3884 [PMID: 16799628 DOI: 10.1038/sj.onc.1209550]
20 **Kolch W**. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J* 2000; **351** Pt 2: 289-305 [PMID: 11023813 DOI: 10.1042/0264-6021]
21 **Roberts LR**, Gores GJ. Hepatocellular carcinoma: molecular pathways and new therapeutic targets. *Semin Liver Dis* 2005; **25**: 212-225 [PMID: 15918149 DOI: 10.1055/s-2005-871200]
22 **Harden TK**, Sondek J. Regulation of phospholipase C isozymes by ras superfamily GTPases. *Annu Rev Pharmacol Toxicol* 2006; **46**: 355-379 [PMID: 16402909 DOI: 10.1146/annurev.pharmtox.46.120604.141223]
23 **To MD**, Perez-Losada J, Mao JH, Balmain A. Crosstalk between Pten and Ras signaling pathways in tumor development. *Cell Cycle* 2005; **4**: 1185-1188 [PMID: 16123588 DOI: 10.4161/cc.4.9.2039]
24 **Huynh H**, Nguyen TT, Chow KH, Tan PH, Soo KC, Tran E. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. *BMC Gastroenterol* 2003; **3**: 19 [PMID: 12906713 DOI: 10.1186/1471-230X-3-19]
25 **Ito Y**, Sasaki Y, Horimoto M, Wada S, Tanaka Y, Kasahara A, Ueki T, Hirano T, Yamamoto H, Fujimoto J, Okamoto E, Hayashi N, Hori M. Activation of mitogen-activated protein kinases/extracellular signal-regulated kinases in human hepatocellular carcinoma. *Hepatology* 1998; **27**: 951-958 [PMID: 9537433 DOI: 10.1002/hep.510270409]
26 **Chung TW**, Lee YC, Kim CH. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. *FASEB J* 2004; **18**: 1123-1125 [PMID: 15132991]
27 **Erhardt A**, Hassan M, Heintges T, Häussinger D. Hepatitis C virus core protein induces cell proliferation and activates ERK, JNK, and p38 MAP kinases together with the MAP kinase phosphatase MKP-1 in a HepG2 Tet-Off cell line. *Virology* 2002; **292**: 272-284 [PMID: 11878930 DOI: 10.1006/viro.2001.1227]
28 **Lee HC**, Tian B, Sedivy JM, Wands JR, Kim M. Loss of Raf kinase inhibitor protein promotes cell proliferation and migration of human hepatoma cells. *Gastroenterology* 2006; **131**: 1208-1217 [PMID: 17030190 DOI: 10.1053/j.gastro.2006.07.012]
29 **Wilhelm SM**, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther* 2008; **7**: 3129-3140 [PMID: 18852116 DOI: 10.1158/1535-7163.MCT-08-0013]
30 **Huynh H**. AZD6244 (ARRY-142886) enhances the antitumor activity of rapamycin in mouse models of human hepatocellular carcinoma. *Cancer* 2010; **116**: 1315-1325 [PMID: 20101735 DOI: 10.1002/cncr.24863]
31 **Choo S**, Ng Q, Chen W, Tham C, Yong W, Wang L, Koh T, Goh B, Thng C, Huynh H, Zee Y, Low L, Toh H. A phase I/II study of AZD6244 in combination with sorafenib in advanced hepatocellular carcinoma. *J Clin Oncol* 2012; **30**: A4100
32 **Zhou Q**, Lui VW, Yeo W. Targeting the PI3K/Akt/mTOR pathway in hepatocellular carcinoma. *Future Oncol* 2011; **7**: 1149-1167 [PMID: 21992728 DOI: 10.2217/fon.11.95]
33 **Alexia C**, Fallot G, Lasfer M, Schweizer-Groyer G, Groyer A. An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. *Biochem Pharmacol* 2004; **68**: 1003-1015 [PMID: 15313394 DOI: 10.1016/j.bcp.2004.05.029]
34 **Desbois-Mouthon C**, Cadoret A, Blivet-Van Eggelpoël MJ,

Bertrand F, Cherqui G, Perret C, Capeau J. Insulin and IGF-1 stimulate the beta-catenin pathway through two signalling cascades involving GSK-3beta inhibition and Ras activation. *Oncogene* 2001; **20**: 252-259 [PMID: 11313952 DOI: 10.1038/sj.onc.1204064]

35 **Hu TH**, Huang CC, Lin PR, Chang HW, Ger LP, Lin YW, Changchien CS, Lee CM, Tai MH. Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. *Cancer* 2003; **97**: 1929-1940 [PMID: 12673720 DOI: 10.1002/cncr.11266]

36 **Villanueva A**, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, Battiston C, Van Laarhoven S, Fiel MI, Di Feo A, Hoshida Y, Yea S, Toffanin S, Ramos A, Martignetti JA, Mazzaferro V, Bruix J, Waxman S, Schwartz M, Meyerson M, Friedman SL, Llovet JM. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1972-183, 1972-183, [PMID: 18929564]

37 **Zhu AX**, Abrams TA, Miksad R, Blaszkowsky LS, Meyerhardt JA, Zheng H, Muzikansky A, Clark JW, Kwak EL, Schrag D, Jors KR, Fuchs CS, Iafrate AJ, Borger DR, Ryan DP. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. *Cancer* 2011; **117**: 5094-5102 [PMID: 21538343 DOI: 10.1002/cncr.26165]

38 **Decaens T**, Luciani A, Itti E, Hulin A, Roudot-Thoraval F, Laurent A, Zafrani ES, Mallat A, Duvoux C. Phase II study of sirolimus in treatment-naive patients with advanced hepatocellular carcinoma. *Dig Liver Dis* 2012; **44**: 610-616 [PMID: 22459565 DOI: 10.1016/j.dld.2012.02.005]

39 **Semela D**, Dufour JF. Angiogenesis and hepatocellular carcinoma. *J Hepatol* 2004; **41**: 864-880 [PMID: 15519663 DOI: 10.1016/j.jhep.2004.09.006]

40 **Mas VR**, Maluf DG, Archer KJ, Yanek KC, Fisher RA. Angiogenesis soluble factors as hepatocellular carcinoma noninvasive markers for monitoring hepatitis C virus cirrhotic patients awaiting liver transplantation. *Transplantation* 2007; **84**: 1262-1271 [PMID: 18049111 DOI: 10.1097/01.tp.0000287596.91520.1a]

41 **Folkman J**. Fundamental concepts of the angiogenic process. *Curr Mol Med* 2003; **3**: 643-651 [PMID: 14601638 DOI: 10.2174/1566524033479465]

42 **El-Assal ON**, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nabika T, Nagasue N. Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. *Hepatology* 1998; **27**: 1554-1562 [PMID: 9620326 DOI: 10.1002/hep.510270613]

43 **Chao Y**, Li CP, Chau GY, Chen CP, King KL, Lui WY, Yen SH, Chang FY, Chan WK, Lee SD. Prognostic significance of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin in patients with resectable hepatocellular carcinoma after surgery. *Ann Surg Oncol* 2003; **10**: 355-362 [PMID: 12734082 DOI: 10.1245/ASO.2003.10.002]

44 **Li XM**, Tang ZY, Zhou G, Lui YK, Ye SL. Significance of vascular endothelial growth factor mRNA expression in invasion and metastasis of hepatocellular carcinoma. *J Exp Clin Cancer Res* 1998; **17**: 13-17 [PMID: 9646228]

45 **Poon RT**, Lau C, Yu WC, Fan ST, Wong J. High serum levels of vascular endothelial growth factor predict poor response to transarterial chemoembolization in hepatocellular carcinoma: a prospective study. *Oncol Rep* 2004; **11**: 1077-1084 [PMID: 15069550]

46 **Yamaguchi R**, Yano H, Iemura A, Ogasawara S, Haramaki M, Kojiro M. Expression of vascular endothelial growth factor in human hepatocellular carcinoma. *Hepatology* 1998; **28**: 68-77 [PMID: 9657098 DOI: 10.1002/hep.510280111]

47 **Uematsu S**, Higashi T, Nouso K, Kariyama K, Nakamura S, Suzuki M, Nakatsukasa H, Kobayashi Y, Hanafusa T, Tsuji T, Shiratori Y. Altered expression of vascular endothelial growth factor, fibroblast growth factor-2 and endostatin in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2005; **20**: 583-588 [PMID: 15836707 DOI: 10.1111/j.1440-1746.2005.03726.x]

48 **El-Assal ON**, Yamanoi A, Ono T, Kohno H, Nagasue N. The clinicopathological significance of heparanase and basic fibroblast growth factor expressions in hepatocellular carcinoma. *Clin Cancer Res* 2001; **7**: 1299-1305 [PMID: 11350898]

49 **Poon RT**, Ng IO, Lau C, Yu WC, Fan ST, Wong J. Correlation of serum basic fibroblast growth factor levels with clinicopathologic features and postoperative recurrence in hepatocellular carcinoma. *Am J Surg* 2001; **182**: 298-304 [PMID: 11587697 DOI: 10.1016/S0002-9610(01)00708-5]

50 **Hisai H**, Kato J, Kobune M, Murakami T, Miyanishi K, Takahashi M, Yoshizaki N, Takimoto R, Terui T, Niitsu Y. Increased expression of angiogenin in hepatocellular carcinoma in correlation with tumor vascularity. *Clin Cancer Res* 2003; **9**: 4852-4859 [PMID: 14581357]

51 **Presta LG**, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 1997; **57**: 4593-4599 [PMID: 9377574]

52 **Siegel AB**, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, Chen H, Clark-Garvey S, Weinberg A, Mandeli J, Christos P, Mazumdar M, Popa E, Brown RS, Rafii S, Schwartz JD. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2992-2998 [PMID: 18565886 DOI: 10.1200/JCO.2007.15.9947]

53 **Hsu CH**, Yang TS, Hsu C, Toh HC, Epstein RJ, Hsiao LT, Chen PJ, Lin ZZ, Chao TY, Cheng AL. Efficacy and tolerability of bevacizumab plus capecitabine as first-line therapy in patients with advanced hepatocellular carcinoma. *Br J Cancer* 2010; **102**: 981-986 [PMID: 20160718 DOI: 10.1038/sj.bjc.6605580]

54 **Sun W**, Sohal D, Haller DG, Mykulowycz K, Rosen M, Soulen MC, Caparro M, Teitelbaum UR, Giantonio B, O'Dwyer PJ, Shaked A, Reddy R, Olthoff K. Phase 2 trial of bevacizumab, capecitabine, and oxaliplatin in treatment of advanced hepatocellular carcinoma. *Cancer* 2011; **117**: 3187-3192 [PMID: 21264839 DOI: 10.1002/cncr.25889]

55 **Thomas MB**, Morris JS, Chadha R, Iwasaki M, Kaur H, Lin E, Kaseb A, Glover K, Davila M, Abbruzzese J. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *J Clin Oncol* 2009; **27**: 843-850 [PMID: 19139433 DOI: 10.1200/JCO.2008.18.3301]

56 **Zhu AX**. Systemic therapy of advanced hepatocellular carcinoma: how hopeful should we be? *Oncologist* 2006; **11**: 790-800 [PMID: 16880238 DOI: 10.1634/theoncologist.11-7-790]

57 **Abou-Alfa GK**, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293-4300 [PMID: 16908937 DOI: 10.1200/JCO.2005.01.3441]

58 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

59 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial.

Lancet Oncol 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

60 **Lee JH**, Chung YH, Kim JA, Shim JH, Lee D, Lee HC, Shin ES, Yoon JH, Kim BI, Bae SH, Koh KC, Park NH. Genetic predisposition of hand-foot skin reaction after sorafenib therapy in patients with hepatocellular carcinoma. *Cancer* 2013; **119**: 136-142 [PMID: 22736425 DOI: 10.1002/cncr.27705]

61 **Abrams TJ**, Lee LB, Murray LJ, Pryer NK, Cherrington JM. SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. *Mol Cancer Ther* 2003; **2**: 471-478 [PMID: 12748309]

62 **Abrams TJ**, Murray LJ, Pesenti E, Holway VW, Colombo T, Lee LB, Cherrington JM, Pryer NK. Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with "standard of care" therapeutic agents for the treatment of breast cancer. *Mol Cancer Ther* 2003; **2**: 1011-1021 [PMID: 14578466]

63 **Murray LJ**, Abrams TJ, Long KR, Ngai TJ, Olson LM, Hong W, Keast PK, Brassard JA, O'Farrell AM, Cherrington JM, Pryer NK. SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. *Clin Exp Metastasis* 2003; **20**: 757-766 [PMID: 14713109]

64 **Zhu AX**, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, Sindhwani V, Blaszkowsky LS, Yoon SS, Lahdenranta J, Bhargava P, Meyerhardt J, Clark JW, Kwak EL, Hezel AF, Miksad R, Abrams TA, Enzinger PC, Fuchs CS, Ryan DP, Jain RK. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol* 2009; **27**: 3027-3035 [PMID: 19470923 DOI: 10.1200/JCO.2008.20.9908]

65 **Faivre S**, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, Zappa M, Lanzalone S, Lin X, Deprimo S, Harmon C, Ruiz-Garcia A, Lechuga MJ, Cheng AL. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. *Lancet Oncol* 2009; **10**: 794-800 [PMID: 19586800 DOI: 10.1016/S1470-2045(09)70171-8]

66 **Bergers G**, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008; **8**: 592-603 [PMID: 18650835 DOI: 10.1038/nrc2442]

67 **Tille JC**, Wood J, Mandriota SJ, Schnell C, Ferrari S, Mestan J, Zhu Z, Witte L, Pepper MS. Vascular endothelial growth factor (VEGF) receptor-2 antagonists inhibit VEGF- and basic fibroblast growth factor-induced angiogenesis in vivo and in vitro. *J Pharmacol Exp Ther* 2001; **299**: 1073-1085 [PMID: 11714897]

68 **Park JW**, Finn RS, Kim JS, Karwal M, Li RK, Ismail F, Thomas M, Harris R, Baudelet C, Walters I, Raoul JL. Phase II, open-label study of brivanib as first-line therapy in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2011; **17**: 1973-1983 [PMID: 21349999 DOI: 10.1158/1078-0432.CCR-10-2011]

69 **Finn RS**, Kang YK, Mulcahy M, Polite BN, Lim HY, Walters I, Baudelet C, Manekas D, Park JW. Phase II, open-label study of brivanib as second-line therapy in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2090-2098 [PMID: 22238246 DOI: 10.1158/1078-0432.CCR-11-1991]

70 **Llovet JM**, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, Kang YK, Assenat E, Lim HY, Boige V, Mathurin P, Fartoux L, Lin DY, Bruix J, Poon RT, Sherman M, Blanc JF, Finn RS, Tak WY, Chao Y, Ezzeddine R, Liu D, Walters I, Park JW. Brivanib in Patients With Advanced Hepatocellular Carcinoma Who Were Intolerant to Sorafenib or for Whom Sorafenib Failed: Results From the Randomized Phase III BRISK-PS Study. *J Clin Oncol* 2013 Aug 26; Epub ahead of print [PMID: 23980090 DOI: 10.1016/S0168-8278(12)61409-3]

71 **Johnson P**, Qin S, Park JW. Brivanib (BRI) versus Sorafenib (SOR) as First-line Therapy in Patients with Unresectable, Advanced Hepatocellular Carcinoma (HCC): Results from the Phase 3 BRISK-FL Study. Boston: 63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2012), 2012: Abstract LB-6

72 **Wood JM**, Bold G, Buchdunger E, Cozens R, Ferrari S, Frei J, Hofmann F, Mestan J, Mett H, O'Reilly T, Persohn E, Rösel J, Schnell C, Stover D, Theuer A, Towbin H, Wenger F, Woods-Cook K, Menrad A, Siemeister G, Schirner M, Thierauch KH, Schneider MR, Drevs J, Martiny-Baron G, Totzke F. PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res* 2000; **60**: 2178-2189 [PMID: 10786682]

73 **Yau T**, Chan P, Pang R, Ng K, Fan ST, Poon RT. Phase 1-2 trial of PTK787/ZK222584 combined with intravenous doxorubicin for treatment of patients with advanced hepatocellular carcinoma: implication for antiangiogenic approach to hepatocellular carcinoma. *Cancer* 2010; **116**: 5022-5029 [PMID: 20629034 DOI: 10.1002/cncr.25372]

74 **Albert DH**, Tapang P, Magoc TJ, Pease LJ, Reuter DR, Wei RQ, Li J, Guo J, Bousquet PF, Ghoreishi-Haack NS, Wang B, Bukofzer GT, Wang YC, Stavropoulos JA, Hartandi K, Niquette AL, Soni N, Johnson EF, McCall JO, Bouska JJ, Luo Y, Donawho CK, Dai Y, Marcotte PA, Glaser KB, Michaelides MR, Davidsen SK. Preclinical activity of ABT-869, a multi-targeted receptor tyrosine kinase inhibitor. *Mol Cancer Ther* 2006; **5**: 995-1006 [PMID: 16648571 DOI: 10.1158/1535-7163.MCT-05-0410]

75 **Toh HC**, Chen PJ, Carr BI, Knox JJ, Gill S, Ansell P, McKeegan EM, Dowell B, Pedersen M, Qin Q, Qian J, Scappaticci FA, Ricker JL, Carlson DM, Yong WP. Phase 2 trial of linifanib (ABT-869) in patients with unresectable or metastatic hepatocellular carcinoma. *Cancer* 2013; **119**: 380-387 [PMID: 22833179 DOI: 10.1002/cncr.27758]

76 **Cainap C**, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens F, Qian J, McKee MD, Ricker JL, Carlson DM, Nowiem SE. Phase III trial of linifanib versus sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2012; **30**: A249

77 **Kanai F**, Yoshida H, Tateishi R, Sato S, Kawabe T, Obi S, Kondo Y, Taniguchi M, Tagawa K, Ikeda M, Morizane C, Okusaka T, Arioka H, Shiina S, Omata M. A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2011; **67**: 315-324 [PMID: 20390419 DOI: 10.1007/s00280-010-1320-2]

78 **Alberts SR**, Fitch TR, Kim GP, Morlan BW, Dakhil SR, Gross HM, Nair S. Cediranib (AZD2171) in patients with advanced hepatocellular carcinoma: a phase II North Central Cancer Treatment Group Clinical Trial. *Am J Clin Oncol* 2012; **35**: 329-333 [PMID: 21422991 DOI: 10.1097/COC.0b013e3182118cdf]

79 **Ciardiello F**, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med* 2008; **358**: 1160-1174 [PMID: 18337605 DOI: 10.1056/NEJMra0707704]

80 **Buckley AF**, Burgart LJ, Sahai V, Kakar S. Epidermal growth factor receptor expression and gene copy number in conventional hepatocellular carcinoma. *Am J Clin Pathol* 2008; **129**: 245-251 [PMID: 18208805 DOI: 10.1309/WF10QAAED3PP93BH]

81 **Zhu AX**, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, Enzinger PC, Bhargava P, Meyerhardt JA, Horgan K, Fuchs CS, Ryan DP. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. *Cancer* 2007; **110**: 581-589 [PMID: 17583545 DOI: 10.1002/cncr.22829]

82 **Huether A**, Hopfner M, Sutter AP, Baradari V, Schuppan D, Scherubl H. Signaling pathways involved in the inhibition of epidermal growth factor receptor by erlotinib in hepatocellular cancer. *World J Gastroenterol* 2006; **12**: 5160-5167 [PMID: 16937526]

83 **Zhu AX**, Rosmorduc O, Evans J, Ross P, Santoro A, Carrilho FJ, Leberre M, Jensen M, Meinhardt G, Kang YK. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with hepatocellular carcinoma (HCC). *Ann Oncol* 2012; **23**: Abstract LBA2

84 **Burris HA**, Hurwitz HI, Dees EC, Dowlati A, Blackwell KL, O'Neil B, Marcom PK, Ellis MJ, Overmoyer B, Jones SF, Harris JL, Smith DA, Koch KM, Stead A, Mangum S, Spector NL. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J Clin Oncol* 2005; **23**: 5305-5313 [PMID: 15955900 DOI: 10.1200/JCO.2005.16.584]

85 **Ramanathan RK**, Belani CP, Singh DA, Tanaka M, Lenz HJ, Yen Y, Kindler HL, Iqbal S, Longmate J, Mack PC, Lurje G, Gandour-Edwards R, Dancey J, Gandara DR. A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. *Cancer Chemother Pharmacol* 2009; **64**: 777-783 [PMID: 19169683 DOI: 10.1007/s00280-009-0927-7]

86 **Golan T**, Javle M. Targeting the insulin growth factor pathway in gastrointestinal cancers. *Oncology* (Williston Park) 2011; **25**: 518-526, 529 [PMID: 21717907]

87 **Pollak MN**, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. *Nat Rev Cancer* 2004; **4**: 505-518 [PMID: 15229476 DOI: 10.1038/nrc1387]

88 **Cariani E**, Lasserre C, Seurin D, Hamelin B, Kemeny F, Franco D, Czech MP, Ullrich A, Brechot C. Differential expression of insulin-like growth factor II mRNA in human primary liver cancers, benign liver tumors, and liver cirrhosis. *Cancer Res* 1988; **48**: 6844-6849 [PMID: 3180092]

89 **De Souza AT**, Hankins GR, Washington MK, Fine RL, Orton TC, Jirtle RL. Frequent loss of heterozygosity on 6q at the mannose 6-phosphate/insulin-like growth factor II receptor locus in human hepatocellular tumors. *Oncogene* 1995; **10**: 1725-1729 [PMID: 7753549]

90 **Chen YW**, Boyartchuk V, Lewis BC. Differential roles of insulin-like growth factor receptor- and insulin receptor-mediated signaling in the phenotypes of hepatocellular carcinoma cells. *Neoplasia* 2009; **11**: 835-845 [PMID: 19724677]

91 **Scharf JG**, Braulke T. The role of the IGF axis in hepatocarcinogenesis. *Horm Metab Res* 2003; **35**: 685-693 [PMID: 14710347]

92 **Higano CS**, Yu EY, Whiting SH, Gordon MS, LoRusso P, Fox F, Katz TL, Roecker JM, Schwartz JD. A phase I, first in man study of weekly IMC-A12, a fully human insulin like growth factor-I receptor IgG1 monoclonal antibody, in patients with advanced solid tumors. *J Clin Oncol* 2007; **25**: s3505

93 **Abou-Alfa GK**, Gansukh B, Chou JF, Shia J, Capanu M, Kalin M, Chen HX, Zojwalla NJ, Katz S, Reidy DL, Kelsen DP, Saltz L. Phase II Study of Cixutumumab (IMC-A12, NSC742460; C) in Hepatocellular Carcinoma. *J Clin Oncol* 2011; **29**: Abstract 4043

94 **Faivre S**, Fartoux L, Bouattour M, Bumsel F, Dreyer C, Raymond E, Rosmorduc O. A phase I study of AVE1642, a human monoclonal antibody-blocking insulin-like growth factor-1 receptor (IGF-1R), given as a single agent and in combination with sorafenib as first-line therapy in patients with advanced hepatocellular carcinoma (HCC). *J Clin Oncol* 2011; **29**: Abstract 270

95 **You WK**, McDonald DM. The hepatocyte growth factor/c-Met signaling pathway as a therapeutic target to inhibit angiogenesis. *BMB Rep* 2008; **41**: 833-839 [PMID: 19123972 DOI: 10.5483/BMBRep.2008.41.12.833]

96 **Yap TA**, de Bono JS. Targeting the HGF/c-Met axis: state of play. *Mol Cancer Ther* 2010; **9**: 1077-1079 [PMID: 20442310 DOI: 10.1158/1535-7163.MCT-10-0122]

97 **Boix L**, Rosa JL, Ventura F, Castells A, Bruix J, Rodés J, Bartrons R. c-met mRNA overexpression in human hepatocellular carcinoma. *Hepatology* 1994; **19**: 88-91 [PMID: 8276372 DOI: 10.1002/hep.1840190115]

98 **Tavian D**, De Petro G, Benetti A, Portolani N, Giulini SM, Barlati S. u-PA and c-MET mRNA expression is co-ordinately enhanced while hepatocyte growth factor mRNA is down-regulated in human hepatocellular carcinoma. *Int J Cancer* 2000; **87**: 644-649 [PMID: 10925356]

99 **Ueki T**, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. *Hepatology* 1997; **25**: 619-623 [PMID: 9049208 DOI: 10.1002/hep.510250321]

100 **Santoro A**, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; **14**: 55-63 [PMID: 23182627 DOI: 10.1016/S1470-2045(12)70490-4]

101 **Herencia C**, Martínez-Moreno JM, Herrera C, Corrales F, Santiago-Mora R, Espejo I, Barco M, Almadén Y, de la Mata M, Rodríguez-Ariza A, Muñoz-Castañeda JR. Nuclear translocation of β-catenin during mesenchymal stem cells differentiation into hepatocytes is associated with a tumoral phenotype. *PLoS One* 2012; **7**: e34656 [PMID: 22506042 DOI: 10.1371/journal.pone.0034656]

102 **Zhang Y**, Wei W, Cheng N, Wang K, Li B, Jiang X, Sun S. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* 2012; **56**: 1631-1640 [PMID: 22610915 DOI: 10.1002/hep.25849]

103 **Srisuttee R**, Koh SS, Kim SJ, Malilas W, Boonying W, Cho IR, Jhun BH, Ito M, Horio Y, Seto E, Oh S, Chung YH. Hepatitis B virus X (HBX) protein upregulates β-catenin in a human hepatic cell line by sequestering SIRT1 deacetylase. *Oncol Rep* 2012; **28**: 276-282 [PMID: 22562294]

104 **Kisseleva T**, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* 2002; **285**: 1-24 [PMID: 12039028 DOI: 10.1016/S0378-1119(02)00398-0]

105 **Bromberg JF**. Activation of STAT proteins and growth control. *Bioessays* 2001; **23**: 161-169 [PMID: 11169589]

106 **Calvisi DF**, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM, Thorgeirsson SS. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* 2006; **130**: 1117-1128 [PMID: 16618406 DOI: 10.1053/j.gastro.2006.01.006]

107 **Jin YJ**, Chung YH, Kim JA, Park WH, Lee D, Seo DD, Ryu SH, Jang MK, Yu E, Lee YJ. Factors predisposing metastatic tumor antigen 1 overexpression in hepatitis B virus associated hepatocellular carcinoma. *Dig Dis Sci* 2012; **57**: 2917-2923 [PMID: 22777614 DOI: 10.1007/s10620-012-2296-z]

108 **Jung SW**, Park NH, Shin JW, Park BR, Kim CJ, Lee JE, Shin ES, Kim JA, Chung YH. Polymorphisms of DNA repair genes in Korean hepatocellular carcinoma patients with chronic hepatitis B: possible implications on survival. *J Hepatol* 2012; **57**: 621-627 [PMID: 22659345 DOI: 10.1016/j.jhep.2012.04.039]

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REVIEW

Endotherapy in chronic pancreatitis

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Abstract

Chronic pancreatitis (CP) is a progressive disease with irreversible changes in the pancreas. Patients commonly present with pain and with exocrine or endocrine insufficiency. All therapeutic efforts in CP are directed towards relief of pain as well as the management of associated complications. Endoscopic therapy offers many advantages in patients with CP who present with ductal calculi, strictures, ductal leaks, pseudocyst or associated biliary strictures. Endotherapy offers a high rate of success with low morbidity in properly selected patients. The procedure can be repeated and failed endotherapy is not a hindrance to subsequent surgery. Endoscopic pancreatic sphincterotomy is helpful in patients with CP with minimal ductal changes while minor papilla sphincterotomy provides relief in patients with pancreas divisum and chronic pancreatitis. Extracorporeal shock wave lithotripsy is the standard of care in patients with large pancreatic ductal calculi. Long term follow up has shown pain relief in over 60% of patients. A transpapillary stent placed across the disruption provides relief in over 90% of patients with ductal leaks. Pancreatic ductal strictures are managed by single large bore stents. Multiple stents are placed for refractory strictures. CP associated benign biliary strictures (BBS) are best treated with multiple plastic stents, as the response to a single plastic stent is poor. Covered self expanding metal stents are increasingly being used in the management of BBS though further long term studies are needed. Pseudocysts are best drained endoscopically with a success rate of 80%-95% at most centers. Endosonography (EUS) has added to the therapeutic armamentarium in the management of patients with CP. Drainage of pseudcysts, cannulation of inaccessible pancreatic ducts and celiac ganglion block in patients with intractable pain are all performed using EUS. Endotherapy should be offered as the first line of therapy in properly selected patients with CP who have failed to respond to medical therapy and require intervention.

Key words: Chronic pancreatitis; Endoscopic retrograde cholangiopancreatography; Pancreatic sphincterotomy; Extracorporeal shockwave lithotripsy; Endosonography

Core tip: Chronic pancreatitis is a challenge to the therapeutic endoscopist. A patient with chronic pancreatitis can present with ductal calculi, leaks, pseudocysts, strictures, pancreatic malignancy or a biliary obstruction. Endoscopic therapy offers a high rate of success in properly selected patients. It offers many advantages over surgery, which for a long time was considered the gold standard in the treatment of chronic pancreatitis. This chapter deals with the management of chronic pancreatitis associated strictures, calculi, leaks and pseudocysts. The role of endosonography in management of pseudocysts, cannulation of inaccessible ducts and pain relief has also been discussed.

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INTRODUCTION

Chronic pancreatitis (CP) is a disease of varied etiology and characterized by progressive and irreversible damage to the pancreas with resultant loss of both endocrine and exocrine functions. Alcohol, smoking, genetic factors and metabolic disorders are common etiological causes[1]. In our country the non alcoholic type of CP is more prevalent[2,3]. Irrespective of the etiology, the majority of patients with CP present with pain as the dominant symptom.

As the disease is irreversible, almost all therapeutic efforts are directed towards control of pain and management of complications associated with CP. For the therapeutic endoscopist, CP is a challenge as patients can present with ductal strictures, calculi, ductal disruption, pseudocysts, biliary strictures, duodenal narrowing or a pancreatic malignancy. Endotherapy is performed in patients with CP who are unlikely to respond or have failed medical therapy as well as to manage the above mentioned complications. Surgery has often been considered the best therapeutic option for patients with CP[4]. However with advances in technology and techniques, endotherapy is offered as first line management in many patients with CP. Endotherapy offers many distinct advantages over surgery. It has a high success rate and low morbidity in properly selected patients. The procedure can be repeated with no extra risk, unlike the morbidity and difficulty associated with repeat surgery. The results are comparable to surgery and failed endotherapy does not hinder subsequent surgery[5-8]. The endoscopic approach can also predict the response to surgical therapy[9]. The endoscopic techniques used are endoscopic retrograde cholangiopancreatography (ERCP) and endosonography (EUS). Extracorporeal shockwave lithotripsy (ESWL) is a part of the endoscopic armamentarium. Advances in EUS have improved therapeutic options, including pseudocyst drainage and cannulation of inaccessible main pancreatic duct (MPD).

In this review, we will discuss the role of endotherapy in diagnosis and management of CP related pancreatic ductal strictures, stones, common bile duct (CBD) strictures and pseudocyst.

ROLE OF ENDOSCOPY IN THE DIAGNOSIS OF CP

ERCP was earlier used both for diagnosis and management of patients with CP. It has sensitivity of 73%-94% and specificity of 90%-100% in visualizing duct related changes in CP[10]. The emergence of magnetic resonance cholangiopancreatography (MRCP) with secretin stimulation, as well as EUS, has minimized the role of ERCP in diagnosing CP. EUS is a better diagnostic modality, especially in early and less advanced CP, as it identifies both ductal and parenchymal changes[11]. EUS has a sensitivity of close to 100% as compared to 80% with ERCP in patients with early CP[12]. MRCP being non-invasive offers a better alternative to ERCP for visualizing ductal changes.

CP WITH MINIMAL DUCTAL CHANGES

Painful CP can occasionally present with minimal or no ductal change in absence of ductal strictures or stones. This is classified as type I CP according to Cremer classification or mild CP of Cambridge classification[13,14]. Endoscopic pancreatic sphincterotomy (EPS) is a documented mode of therapy and offers symptomatic relief in some of these patients. Both the standard pull type and the needle knife sphincterotomy over a stent can be performed. A 64% relief in pain on follow up of 6.5 years has been reported following EPS[15]. High success rates of 98% and low complication rates of around 4% have been reported on retrospective analysis[16]. Randomized studies have shown a higher incidence of pancreatitis in high risk patients following pull type sphincterotomy as compared to the needle knife technique[17]. Most workers report an incidence of around 12% for post EPS pancreatitis. Placement of a nasopancreatic tube (NPT) or pancreatic stent can reduce this incidence significantly[18]. Restenosis is reported in around 14% of patients on long term follow up[19]. It is believed that restenosis is less common following the longer incision with the pull type as compared to needle knife technique[20]. The presence of periductal fibrosis seen in patients with CP may lower the incidence of post procedure pancreatitis. An additional biliary sphincterotomy is only indicated in the following conditions[21]: (1) presence of cholangitis; (2) CBD > 12 mm diameter; (3) serum alkaline phosphates > 2 times upper limit of normal; and (4) difficult access to MPD.

Minor papilla sphincterotomy

Minor papilla sphincterotomy (MiES) was first performed by Cotton[22]. It is indicated in those patients with CP with minimal ductal changes who have a pancreas divisum or a dominant dorsal duct. Both the pull type and needle knife technique can be used. The evidence of any definite benefit from MiES is debatable as studies include small numbers of heterogeneous patients and are not conclusive. Significant pain relief on a 2-year follow up has been reported following MiES and stenting of patients with CP[23]. Relief of pain is also seen in 41% of patients with CP following MiES as compared to 77% with acute recurrent pancreatitis or 33% of patients with CP with no pain[24]. Post ERCP pancreatitis has been reported in up to 15% of patients[25] and restenosis was seen in 20%-24% on a 6-year follow up[26].

ENDOSCOPIC MANAGEMENT OF PANCREATIC DUCTAL STRICTURES

Strictures of MPD are frequently seen as a consequence of CP and could be due to inflammation or fibrosis. In our experience of 1000 patients who underwent ESWL, the incidence of strictures was 18%[2]. MPD strictures are defined as a high grade narrowing of MPD with one of the following[9,27]: (1) MPD dilatation > 6 mm beyond

the stricture; (2) failure of contrast to flow alongside the stricture or 6 Fr NPT; and (3) presence of pain during continuous perfusion of the NPT with normal saline for 24 h.

Endotherapy is ideal for single strictures in the head while isolated strictures in the tail or multiple strictures with a chain of lake appearance are not amenable to endotherapy[9]. Prior to stent placement tight strictures need to be dilated with Teflon bougies, Sohendra stent retriever or a balloon dilator[9,27]. Large bore stents 7-10 Fr should be deployed as they have longer patency[27]. Delhaye *et al*[27] followed a protocol where a single stent was placed across a stricture and exchanged every 6 mo or when the patient was symptomatic. Stents were placed for 24 mo. Patients were restented if symptoms recurred. Surgery was considered if patients responded to stent placement but needed frequent or repeated stenting. Cumulative data from several workers revealed pain relief between 70%-94% for a single pancreatic stent on follow up of 14-69 mo[9]. Recurrence of strictures was reported in 38% of patients after 2 years follow up[28]. The concept of multiple plastic stenting for MPD strictures not responding to a single stent placement was advocated by Costamagna *et al*[29]. In their study, after removal of a single stent, the stricture was dilated and multiple plastic stents 8.5-11.5 Fr diameter were placed. A mean of 3 stents were used. The stents were removed 12 mo later. Stricture resolution was seen in 95% and pain relief in 84% on a 38 mo follow up.

Complications with pancreatic stenting can occur. Occlusion was seen with the passage of time and migration was present in 10% of patients[30]. Distal migration and impaction on the opposite duodenal wall can cause perforation while proximal migration into the pancreas is a technical challenge for the endoscopist. The possibility of stent induced fibrosis has raised concerns[31]. However with the preexisting fibrosis of MPD there has been no significant clinical impact. The search for an ideal pancreatic stent continues and a new "wing stent" to prevent clogging as well as an "S" shaped stent to prevent migration are undergoing evaluation[32,33]. The use of covered metal stents (CSEMS) for pancreatic strictures is also under evaluation. The initially used CSEMS had the disadvantage of stent migration. Subsequently a new "bumpy stent" has been tried for MPD strictures in 32 patients[34,35]. The stent had antimigratory properties and its contours adapted to the MPD. These were extracted at 3 mo and were effective in resolving the MPD strictures. However they were associated with the formation of de novo strictures and further trials are needed to evaluate their long term efficacy and safety.

European Society of Gastrointestinal Endoscopy (ESGE) guidelines state that dominant PD strictures be treated by placing a single 10 Fr stent with stent exchanges planned for 1 year. Multiple plastic stents should be deployed in a stricture which persists after 1 year of single stent placement[36]. Uncovered SEMS should not be placed in MPD. ESGE guidelines also state that temporary placement of fully covered SEMS should only performed in the setting of trials[36].

ENDOTHERAPY OF PANCREATIC DUCTAL CALCULI

Pancreatic ductal calculi are a consequence of CP and tend to aggravate or produce pain by obstructing pancreatic ducts and producing upstream hypertension. They can occur in 50% of patients with CP[8]. Stones seen in the tropics and of the non-alcoholic type of CP tend to be larger and denser than those seen in the alcoholic variety[37,38]. The large size could also be due to delay in reporting for therapy[2]. Stones > 5 mm in size can usually be extracted with a Dormia basket, or balloon trawl following EPS. However stones > 5 mm in size are often impacted and difficult to extract by the standard techniques[2,37,39]. Large calculi need to be fragmented prior to extraction or spontaneous expulsion from the MPD. ESWL is now accepted as the standard of care in the management of large PD calculi not amenable to routine endotherapy[2,36,37,40-45]. ESWL is very effective in fragmenting both radio-opaque and radio-lucent calculi in the MPD. A meta-analysis of 17 studies with a total of 491 patients revealed a clearance rate between 37%-100% and good pain relief[46]. Another review of 11 studies with over 1100 patients showed successful stone fragmentation in 89%[47]. Our own single center study of over 1000 patients shows complete clearance in 76% patients and partial clearance in another 17% patients following ESWL and endotherapy for large calculi[2] (Figures 1 and 2).

The following protocol is followed at our center for patients with large PD calculi[2]. Patients with large calculi in the head or body and with pain as the main complaint are subjected to ESWL. Patients with isolated calculi in the tail, multiple MPD strictures, extensive calculi in head, body and tail, associated head mass, pseudocysts and pregnancy are excluded from ESWL. The procedure is performed with a Ⅲ generation electromagnetic lithotripter with bi-dimensional fluoroscopy and ultrasound targeting facility. (Delta compact-Dornier MedTech Wessling Germany). Epidural anesthesia is preferred in most patients[48]. It is effective and offers many advantages as reported in our study of over 1500 patients. Radio-opaque calculi are subjected to ESWL under fluoroscopic guidance. For radio-lucent calculi, a NPT is placed and contrast is passed through this tube to help localize the calculi. The aim of fragmentation is to break the calculi to 3 mm or less to facilitate to their extraction or expulsion[2,49]. An average of 3 sessions is generally required (5000-6000 shocks per session). The protocol is shown in Figure 3. A few studies have advocated use of ESWL alone followed by spontaneous expulsion of fragments[50]. A randomized controlled trial of 55 patients compared results of ESWL and ERCP with ESWL alone. The only difference was higher cost and longer stay in the ESWL and ERCP group[51]. At our center, we prefer to extract

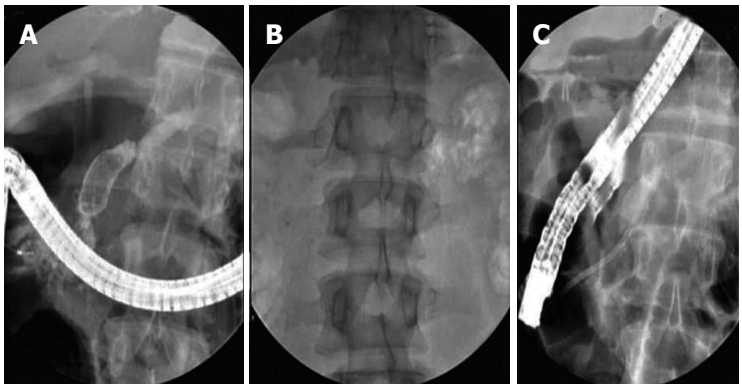

Figure 1 Large pancreatic calculi in head (A), genu in a patient with pancreas divisum (B) and chronic pancreatitis cleared by extracorporeal shockwave lithotripsy followed by pancreatic stenting[49] (C).

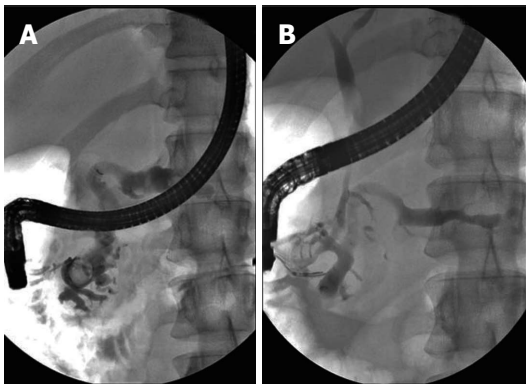

Figure 2 Large pancreatic calculi in head. Post extracorporeal shockwave lithotripsy reduction in diameter of main pancreatic duct[49] (A, B).

the fragments from the MPD by ERCP following the ESWL procedure as fragments tend to be denser and adherent and do not clear spontaneously[2,49].

Short term pain relief following ESWL was seen in 84% of our patients[2] and similar results have been reported by others[39]. Very few long term follow up studies are available. Two-thirds of patients were found to be pain free on long term follow up[8,52]. A recent study showed pain relief in 85%, complete pain relief with no narcotic use in 50% and avoidance of surgery in 84% of 120 patients on long term follow up after ESWL[53]. Our own data on long term follow up is encouraging and over 60% of patients are pain free on follow up of more than 5 years[54]. In conclusion, in properly selected group of patients with large PD calculi, ESWL is a useful tool and provides adequate long term pain relief. A few patients also benefit in exocrine and endocrine dysfunction though the numbers are too small to be significant[54]. ESWL is a safe procedure and well tolerated. Minor side effects such as transient pain and bruising of skin at the site of shock delivery have been described[2,37,49]. The incidence of pancreatitis is not higher following ESWL.

Other techniques for extraction of large PD calculi include intraductal laser or electro hydraulic lithotripsy through a pancreatoscope or spyscope[55,56]. Experience with these modalities is small and success rates are discordant. These procedures are technically difficult and require non standard equipment. At present, they are only to be considered as second line management after failed ESWL[36].

CHRONIC PANCREATITIS RELATED BENIGN BILIARY STRICTURES

CBD strictures occur in 3%-46% of patients with CP[30]. Strictures can be reversible due to inflammation or compression with a pseudocyst. They are irreversible following fibrosis. ESGE guidelines recommend treating CP related benign biliary strictures (BBS) in cases with symptoms, secondary biliary cirrhosis, biliary stones, asymptomatic elevation of serum alkaline phosphates > 2-3 times upper limit of normal or raised serum bilirubin persisting for over 1 mo[36]. Placement of a single plastic stent in the CBD is associated with poor success rates. Long term results have disappointing and sustained benefit is seen in around 25% of patients on follow up of 46 mo[57]. Single plastic stents are associated with poor resolution and higher relapse rate. The presence of pancreatic head calcification is an important factor for failure of this therapy[58]. Placement of multiple plastic stents in CP related BBS is technically successful in over 95% of patients and offers the best results. Complete therapy requires approximately four ERCP procedures and stents exchanges performed every 3 mo for 1 year. Single stents provided relief in 31% of 350 patients as compared to 62% in 50 patients who received multiple stents[36]. Catalano *et al*[59] performed a non-randomized study comparing single and multiple plastic stents in CP related BBS. Clinically, success was reported in 92% with multiple stents as compared to 24% with single stents. Uncovered SEMS for BBS are not advocated and partially or fully covered SEMS have been used with a success rate of 50%-80% on follow up for 22-28 mo[60,61]. A recently conducted multicenter trial using fully covered SEMS (FCSEMS) in BBS included 127 patients of CP. It concluded that FCSEMS may be useful for treatment of BBS particularly in patients with CP[62]. There has been no head to head study comparing single or multiple plastic stents and metal stents in BBS due to CP and surgery. The choice and option of surgery depends upon patient preference, expertise at the treating center and the presence of co morbidities such as cirrhosis or collaterals.

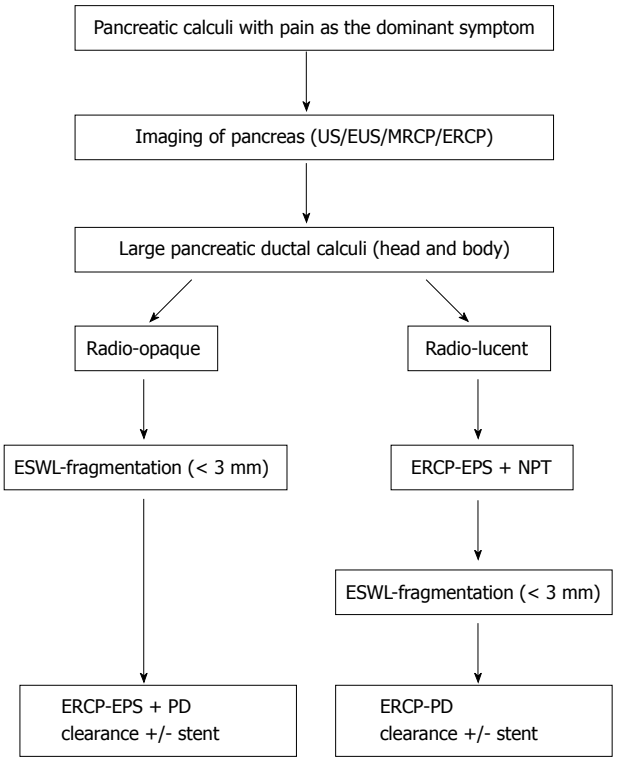

Figure 3 Protocol followed for extracorporeal shockwave lithotripsy in chronic calcific pancreatitis. NPT: Naso pancreatic tube; EPS: Endoscopic pancreatic sphincterotomy; ERCP: Endoscopic retrograde cholangiopancreatography; ESWL: Extracorporeal shockwave lithotripsy; US: Ultrasonography; PD: Pancreatic duct; EUS: Endosonography; MRCP: Magnetic resonance cholangiopancreatography.

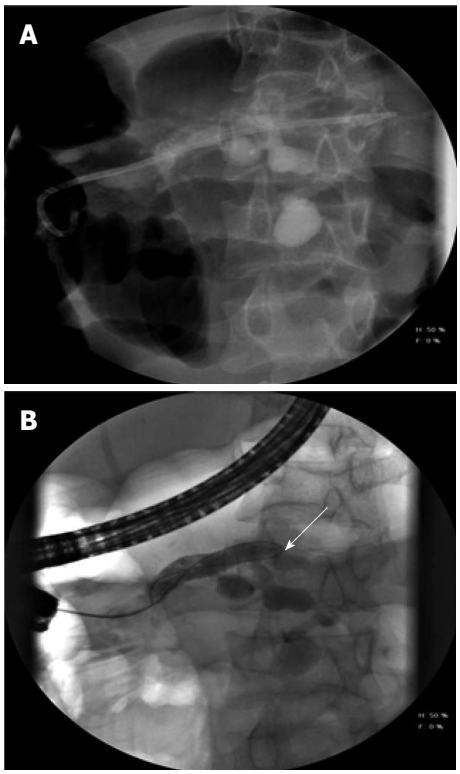

Figure 4 Mid body leak (arrow) with extravasated contrast in a patient with chronic pancreatitis (A) and dilated pancreatic duct (B). Stent placed across the leak.

PANCREATIC DUCTAL LEAKS

Leaks from the MPD or side branches can occur following blow out of the ducts due to obstruction by stone or strictures. PD leak is defined as extravasation of contrast material from the ductal system at ERCP[63]. Disruption may be partial or complete and leads to fluid collection, pseudocysts, ascites, pleural effusion and external or internal fistulas[9,27]. Placement of transpapillary stents offers the best treatment in patients with PD disruption as it converts the high pressure ductal system into a low pressure one with preferential flow across the stents[27]. Resolution of leak was seen in 92% of patients when the stent bridged the disruption, 50% when placed proximal to the disruption and 44% when a short transpapillary stent was placed[63] (Figure 4). In patients with complete transection where stenting is not feasible a multidisciplinary approach with a help of interventional radiologist or the surgeon may be required.

ENDOSCOPY OF PSEUDOCYSTS

Pancreatic pseudocyst (PPC) in CP is the result of disruption of the MPD or its side branches and occurs in 20%-40% of patients[64]. Disruption generally follows obstruction by stones or strictures. Treatment is indicated for symptomatic PPC or those which increase in size. Symptoms result due to compression of adjacent structures or due to infection. It has also been suggested that prophylactic treatment be performed in certain specific situations to prevent complications. These include Pancreatic-pleural fistula fistula, cysts > 5 mm lasting for over 6 wk, compression of major vessels or presence of large pancreatic stones in MPD[65]. There is generally a low rate of spontaneous resolution of PPC in patients with CP though small asymptomatic cysts can be followed up[66]. Drainage of pseudocysts can be transmural (transgastric or transduodenal) or transpapillary. Transmural drainage is ideal for PPC which bulge into the lumen of stomach or duodenum. Transduodenal drainage offers the best success when compared to transgastric drainage[67]. This is because cystoduodenal fistulas tend to remain patent longer than cystogastric fistulas. Placement of one or more pig tailed stents is better when compared to straight stents. Straight stents are associated with a higher rate of bleed (around 7%) as well as migration[68]. Stents should be left in place for a longer duration as their removal within 2 mo is associated with a higher incidence of PPC recurrence[36]. Pseudoaneurysm can complicate management of PPC because of the associated haemorrhage and consequent high mortality[69]. Delhaye *et al*[27] recommend prophylactic embolization of pseudoaneurysms prior to drainage of an adjacent PPC.

Transpapillary drainage is reserved for small cysts (< 6 cm size) and those in communication with the MPD. The role of EUS guided drainage for nonbulging PPC

will be discussed in the next section. Comparison of EUS guided drainage with surgery in an RCT revealed that endoscopic drainage was significantly better than surgery in terms of cost and length of stay over a 3 mo follow up[70]. Complications include bleed, infection and leak of around 4% each with a mortality of 0.5%[71]. Infection is more likely with transpapillary drainage and leak is more likely with transmural drainage. Routine antibiotic administration is recommended for drainage of PPC[72]. With a success rate of 80%-95% at most centers, a recurrence rate of 10%-20% and results comparative or better than surgery, endoscopy is the preferred first line of management for patients with PPC in the background of CP[27,36].

ENDOSONOGRAPHY IN CP

EUS is an excellent diagnostic modality especially in patients with early CP. It also has a definite therapeutic role in the following situations and these are discussed briefly.

PPC drainage

EUS is ideal for drainage of nonbulging PPC and cysts as far as 4 cm from the stomach or duodenal wall have been drained[73]. Around 44%-53% of PPCs belong to this category. In the presence of collaterals the site of drainage is better identified with EUS, thus making the procedure safer. The complication rate is however similar when PPCs are drained with or without EUS guidance[74]. A recent randomized trial comparing EUS guided and surgical cystogastrostomy for pseudocysts revealed shorter hospital stay, lower cost and better physical and mental health in the endoscopy group. None in the endoscopy group had pseudocyst recurrence and therapy was successful in all the patients[75].

EUS guided access of MPD

EUS guided access or drainage is indicated following failed conventional drainage of MPD. It can be *via* the stomach (pancreatogastric) or duodenum (pancreatobulbar). The duodenal route is preferred because of better stability of the EUS scope[9]. A guidewire can be passed into the duodenum for a rendezvous procedure or transmural drainage can be performed. Success rates of 77%-92% have been reported[76,77]. Complications include pain, bleeding, perforation and hematoma and morbidity of 0%-44% has been reported[76-78]. EUS guided access of the MPD is a technically challenging procedure and should always be performed by experts and under radiological guidance[9].

EUS guided celiac block

Patients who have failed to respond to intensive medical or endoscopic therapy and are not candidates suitable for surgery can be provided relief from pain by EUS guided celiac block. A combination of corticosteroids (triamcinolone) and anesthetic agents (bupivacaine) is injected in and around the celiac plexus under EUS guidance. A recent meta analysis has reported pain relief in 50%-55% of patients though the pain relief is transient[79,80]. Patients who are younger than 45 years or have previous pancreatic surgery are less likely to benefit[81]. EUS guided celiac block is shown to be superior to fluoroscopy guided celiac block for pain relief and pain preference in our study[82]. EUS guided nerve block can produce diarrhea, hypertension due to sympathetic blockade and unopposed parasympathetic activity[11,80].

CONCLUSION

In conclusion, management of CP is a multidisciplinary task involving the physician, endoscopist, interventional radiologist and surgeon. Their roles are complementary to each other. As mentioned earlier endotherapy is effective, less invasive than surgery, offers good results and is associated with low morbidity and mortality. It can be repeated and does not interfere with any subsequent surgical procedure. It is therefore advisable to offer endotherapy as the first line treatment in properly selected patients with CP.

REFERENCES

1 **Lankisch PG**, Banks PA, editors. Pancreatitis, New York. Berlin: Springer-Verlag, 1998: 199 [DOI: 10.1007/978-3-642-80320-8_13]

2 **Tandan M**, Reddy DN, Santosh D, Vinod K, Ramchandani M, Rajesh G, Rama K, Lakhtakia S, Banerjee R, Pratap N, Venkat Rao G. Extracorporeal shock wave lithotripsy and endotherapy for pancreatic calculi-a large single center experience. *Indian J Gastroenterol* 2010; **29**: 143-148 [PMID: 20717860 DOI: 10.1007/s12664-010-0035-y]

3 **Balakrishnan V**, Unnikrishnan AG, Thomas V, Choudhuri G, Veeraraju P, Singh SP, Garg P, Pai CG, Devi RN, Bhasin D, Jayanthi V, Premalatha N, Chacko A, Kar P, Rai RR, Rajan R, Subhalal N, Mehta R, Mishra SP, Dwivedi M, Vinayakumar KR, Jain AK, Biswas K, Mathai S, Varghese J, Ramesh H, Alexander T, Philip J, Raj VV, Vinodkumar A, Mukevar S, Sawant P, Nair P, Kumar H, Sudhindran S, Dhar P, Sudheer OV, Sundaram KR, Tantri BV, Singh D, Nath TR. Chronic pancreatitis. A prospective nationwide study of 1,086 subjects from India. *JOP* 2008; **9**: 593-600 [PMID: 18762690]

4 **Cahen DL**, Gouma DJ, Nio Y, Rauws EA, Boermeester MA, Busch OR, Stoker J, Laméris JS, Dijkgraaf MG, Huibregtse K, Bruno MJ. Endoscopic versus surgical drainage of the pancreatic duct in chronic pancreatitis. *N Engl J Med* 2007; **356**: 676-684 [PMID: 17301298 DOI: 10.1056/NEJMoa060610]

5 **Bradley EL**. Long term results of pancreaticojejunostomy in patients with chronic pancreatitis. *Am J Surg* 1987; **153**: 207-213 [DOI: 10.1016/0002-9610(87)90816-6]

6 **Cremer M**, Devière J, Delhaye M, Baize M, Vandermeeren A. Stenting in severe chronic pancreatitis: results of medium-term follow-up in seventy-six patients. *Endoscopy* 1991; **23**: 171-176 [PMID: 1860448 DOI: 10.1055/s-2007-1010649]

7 **Farnbacher MJ**, Schoen C, Rabenstein T, Benninger J, Hahn EG, Schneider HT. Pancreatic duct stones in chronic pancreatitis: criteria for treatment intensity and success. *Gastrointest Endosc* 2002; **56**: 501-506 [PMID: 12297764 DOI: 10.1016/S0016-5107(02)70433-1]

8 **Rösch T**, Daniel S, Scholz M, Huibregtse K, Smits M, Schneider T, Ell C, Haber G, Riemann JF, Jakobs R, Hintze R, Adler A, Neuhaus H, Zavoral M, Zavada F, Schusdziarra V, Soehendra N. Endoscopic treatment of chronic pancreatitis: a multicenter study of 1000 patients with long-term follow-

up. *Endoscopy* 2002; **34**: 765-771 [PMID: 12244496 DOI: 10.1055/s-2002-34256]
9 **Tringali A**, Boskoski I, Costamagna G. The role of endoscopy in the therapy of chronic pancreatitis. *Best Pract Res Clin Gastroenterol* 2008; **22**: 145-165 [PMID: 18206819 DOI: 10.1016/j.bpg.2007.10.021]
10 **Enríquez WK**. Diagnostic and therapeutic endoscopy of pancreas and biliary tract. *Rev Gastroenterol Mex* 2006; **71** Suppl 1: 36-38 [PMID: 17037767]
11 **Attasaranya S**, Abdel Aziz AM, Lehman GA. Endoscopic management of acute and chronic pancreatitis. *Surg Clin North Am* 2007; **87**: 1379-1402, viii [PMID: 18053837 DOI: 10.1016/j.suc.2007.08.006]
12 **Kahl S**, Glasbrenner B, Leodolter A, Pross M, Schulz HU, Malfertheiner P. EUS in the diagnosis of early chronic pancreatitis: a prospective follow-up study. *Gastrointest Endosc* 2002; **55**: 507-511 [PMID: 11923762 DOI: 10.1067/mge.2002.122610]
13 **Cremer M**, Toussaint J, Hermanus A, Deltenre M, De Tceuf J, Engelholm L. Primary chronic pancreatitis. A classification based on endoscopic pancreatography (author's transl). *Acta Gastroenterol Belg* 1976; **39**: 522-546 [PMID: 1023728]
14 **Sarner M**, Cotton PB. Classification of pancreatitis. *Gut* 1984; **25**: 756-759 [PMID: 6735257 DOI: 10.1136/gut.25.7.756]
15 **Gabbrielli A**, Mutignani M, Pandolfi M, Perri V, Costamagna G. Endotherapy of early onset idiopathic chronic pancreatitis: results with long-term follow-up. *Gastrointest Endosc* 2002; **55**: 488-493 [PMID: 11923759 DOI: 10.1067/mge.2002.122651]
16 **Ell C**, Rabenstein T, Schneider HT, Ruppert T, Nicklas M, Bulling D. Safety and efficacy of pancreatic sphincterotomy in chronic pancreatitis. *Gastrointest Endosc* 1998; **48**: 244-249 [PMID: 9744598 DOI: 10.1016/S0016-5107(98)70185-3]
17 **Varadarajulu S**, Wilcox CM. Randomized trial comparing needle-knife and pull-sphincterotome techniques for pancreatic sphincterotomy in high-risk patients. *Gastrointest Endosc* 2006; **64**: 716-722 [PMID: 17055863 DOI: 10.1016/j.gie.2006.02.058]
18 **Hookey LC**, RioTinto R, Delhaye M, Baize M, Le Moine O, Devière J. Risk factors for pancreatitis after pancreatic sphincterotomy: a review of 572 cases. *Endoscopy* 2006; **38**: 670-676 [PMID: 16810591 DOI: 10.1055/s-2006-925447]
19 **Kozarek RA**, Ball TJ, Patterson DJ, Brandabur JJ, Traverso LW, Raltz S. Endoscopic pancreatic duct sphincterotomy: indications, technique, and analysis of results. *Gastrointest Endosc* 1994; **40**: 592-598 [PMID: 7988825 DOI: 10.1016/S0016-5107(94)70260-8]
20 **Siegel JH**, Cohen SA. Pull or push pancreatic sphincterotomy for sphincter of Oddi dysfunction? A conundrum for experts only. *Gastrointest Endosc* 2006; **64**: 723-725 [PMID: 17055864 DOI: 10.1016/j.gie.2006.04.018]
21 **Kim MH**, Myung SJ, Kim YS, Kim HJ, Seo DW, Nam SW, Ahn JH, Lee SK, Min YI. Routine biliary sphincterotomy may not be indispensable for endoscopic pancreatic sphincterotomy. *Endoscopy* 1998; **30**: 697-701 [PMID: 9865559 DOI: 10.1055/s-2007-1001391]
22 **Cotton PB**. Duodenoscopic papillotomy at the minor papilla for recurrent dorsal pancreatitis. *Endosc Dig* 1978; **3**: 27-28
23 **Vitale GC**, Vitale M, Vitale DS, Binford JC, Hill B. Long-term follow-up of endoscopic stenting in patients with chronic pancreatitis secondary to pancreas divisum. *Surg Endosc* 2007; **21**: 2199-2202 [PMID: 17514389 DOI: 10.1007/s00464-007-9347-0]
24 **Watkins JL**, Lehman GA. Minor Papilla Endscopic Sphincterotomy. In: Baron TH, Kozarrek R, Carr-Lock DL. ERCP. Amsterdam : Elseiver, 2008; 143-157
25 **Lehman GA**, Sherman S, Nisi R, Hawes RH. Pancreas divisum: results of minor papilla sphincterotomy. *Gastrointest Endosc* 1993; **39**: 1-8 [PMID: 8454127 DOI: 10.1016/S0016-5107(93)70001-2]
26 **Attwell A**, Borak G, Hawes R, Cotton P, Romagnuolo J. Endoscopic pancreatic sphincterotomy for pancreas divisum by using a needle-knife or standard pull-type technique: safety and reintervention rates. *Gastrointest Endosc* 2006; **64**: 705-711 [PMID: 17055861 DOI: 10.1016/j.gie.2006.02.057]
27 **Delhaye M**, Matos C, Devière J. Endoscopic technique for the management of pancreatitis and its complications. *Best Pract Res Clin Gastroenterol* 2004; **18**: 155-181 [PMID: 15123090 DOI: 10.1016/S1521-6918(03)00077-5]
28 **Eleftherladis N**, Dinu F, Delhaye M, Le Moine O, Baize M, Vandermeeren A, Hookey L, Devière J. Long-term outcome after pancreatic stenting in severe chronic pancreatitis. *Endoscopy* 2005; **37**: 223-230 [PMID: 18556820 DOI: 10.1055/s-2005-860988]
29 **Costamagna G**, Bulajic M, Tringali A, Pandolfi M, Gabbrielli A, Spada C, Petruzziello L, Familiari P, Mutignani M. Multiple stenting of refractory pancreatic duct strictures in severe chronic pancreatitis: long-term results. *Endoscopy* 2006; **38**: 254-259 [PMID: 16528652 DOI: 10.1055/s-2005-921069]
30 **Adler DG**, Lichtenstein D, Baron TH, Davila R, Egan JV, Gan SL, Qureshi WA, Rajan E, Shen B, Zuckerman MJ, Lee KK, VanGuilder T, Fanelli RD. The role of endoscopy in patients with chronic pancreatitis. *Gastrointest Endosc* 2006; **63**: 933-937 [PMID: 16733106 DOI: 10.1016/j.gie.2006.02.003]
31 **Kozarek RA**. Pancreatic stents can induce ductal changes consistent with chronic pancreatitis. *Gastrointest Endosc* 1990; **36**: 93-95 [PMID: 2335298 DOI: 10.1016/S0016-5107(90)70958-3]
32 **Raju GS**, Gomez G, Xiao SY, Ahmed I, Brining D, Bhutani MS, Kalloo AN, Pasricha PJ. Effect of a novel pancreatic stent design on short-term pancreatic injury in a canine model. *Endoscopy* 2006; **38**: 260-265 [PMID: 16528653]
33 **Ishihara T**, Yamaguchi T, Seza K, Tadenuma H, Saisho H. Efficacy of s-type stents for the treatment of the main pancreatic duct stricture in patients with chronic pancreatitis. *Scand J Gastroenterol* 2006; **41**: 744-750 [PMID: 16716976 DOI: 10.1080/00365520500383597]
34 **Park do H**, Kim MH, Moon SH, Lee SS, Seo DW, Lee SK. Feasibility and safety of placement of a newly designed, fully covered self-expandable metal stent for refractory benign pancreatic ductal strictures: a pilot study (with video). *Gastrointest Endosc* 2008; **68**: 1182-1189 [PMID: 19028228 DOI: 10.1016/j.gie.2008.07.027]
35 **Moon SH**, Kim MH, Park do H, Song TJ, Eum J, Lee SS, Seo DW, Lee SK. Modified fully covered self-expandable metal stents with antimigration features for benign pancreatic-duct strictures in advanced chronic pancreatitis, with a focus on the safety profile and reducing migration. *Gastrointest Endosc* 2010; **72**: 86-91 [PMID: 20493483 DOI: 10.1016/j.gie.2010.01.063]
36 **Dumonceau JM**, Delhaye M, Tringali A, Dominguez-Munoz JE, Poley JW, Arvanitaki M, Costamagna G, Costea F, Devière J, Eisendrath P, Lakhtakia S, Reddy N, Fockens P, Ponchon T, Bruno M. Endoscopic treatment of chronic pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2012; **44**: 784-800 [PMID: 22752888 DOI: 10.1055/s-0032-1309840]
37 **Ong WC**, Tandan M, Reddy V, Rao GV, Reddy N. Multiple main pancreatic duct stones in tropical pancreatitis: safe clearance with extracorporeal shockwave lithotripsy. *J Gastroenterol Hepatol* 2006; **21**: 1514-1518 [PMID: 16928210 DOI: 10.1111/j.1440-1746.2006.04224.x]
38 **Chari S**, Jayanthi V, Mohan V, Malathi S, Madanagopalan N, Viswanathan M. Radiological appearance of pancreatic calculi in tropical versus alcoholic chronic pancreatitis. *J Gastroenterol Hepatol* 1992; **7**: 42-44 [PMID: 1543866 DOI: 10.1111/j.1440-1746.1992.tb00932.x]
39 **Lehman GA**. Role of ERCP and other endoscopic modalities in chronic pancreatitis. *Gastrointest Endosc* 2002; **56**: S237-S240 [PMID: 12447274 DOI: 10.1016/S0016-5107(02)70018-7]
40 **Sauerbruch T**, Holl J, Sackmann M, Paumgartner G. Ex-

tracorporeal lithotripsy of pancreatic stones in patients with chronic pancreatitis and pain: a prospective follow up study. *Gut* 1992; **33**: 969-972 [PMID: 1644340 DOI: 10.1136/gut.33.7.969]
41 **Delhaye M**, Vandermeeren A, Baize M, Cremer M. Extracorporeal shock-wave lithotripsy of pancreatic calculi. *Gastroenterology* 1992; **102**: 610-620 [PMID: 1732129]
42 **Dumonceau JM**, Devière J, Le Moine O, Delhaye M, Vandermeeren A, Baize M, Van Gansbeke D, Cremer M. Endoscopic pancreatic drainage in chronic pancreatitis associated with ductal stones: long-term results. *Gastrointest Endosc* 1996; **43**: 547-555 [PMID: 8781931 DOI: 10.1016/S0016-5107(96)70189-X]
43 **Costamagna G**, Gabbrielli A, Mutignani M, Perri V, Pandolfi M, Boscaini M, Crucitti F. Extracorporeal shock wave lithotripsy of pancreatic stones in chronic pancreatitis: immediate and medium-term results. *Gastrointest Endosc* 1997; **46**: 231-236 [PMID: 9378210 DOI: 10.1016/S0016-5107(97)70092-0]
44 **Neuhaus H**. Fragmentation of pancreatic stones by extracorporeal shock wave lithotripsy. *Endoscopy* 1991; **23**: 161-165 [PMID: 1860446 DOI: 10.1055/s-2007-1010647]
45 **Kozarek RA**, Brandabur JJ, Ball TJ, Gluck M, Patterson DJ, Attia F, France R, Traverso LW, Koslowski P, Gibbons RP. Clinical outcomes in patients who undergo extracorporeal shock wave lithotripsy for chronic calcific pancreatitis. *Gastrointest Endosc* 2002; **56**: 496-500 [PMID: 12297763 DOI: 10.1016/S0016-5107(02)70432-X]
46 **Guda NM**, Partington S, Freeman ML. Extracorporeal shock wave lithotripsy in the management of chronic calcific pancreatitis: a meta-analysis. *JOP* 2005; **6**: 6-12 [PMID: 15650279]
47 **Nguyen-Tang T**, Dumonceau JM. Endoscopic treatment in chronic pancreatitis, timing, duration and type of intervention. *Best Pract Res Clin Gastroenterol* 2010; **24**: 281-298 [PMID: 20510829 DOI: 10.1016/j.bpg.2010.03.002]
48 **Darisetty S**, Tandan M, Reddy DN, Kotla R, Gupta R, Ramchandani M, Lakhtakia S, Rao GV, Banerjee R. Epidural anesthesia is effective for extracorporeal shock wave lithotripsy of pancreatic and biliary calculi. *World J Gastrointest Surg* 2010; **2**: 165-168 [PMID: 21160867 DOI: 10.4240/wjgs.v2.i5.165]
49 **Tandan M**, Reddy DN. Extracorporeal shock wave lithotripsy for pancreatic and large common bile duct stones. *World J Gastroenterol* 2011; **17**: 4365-4371 [PMID: 22110261 DOI: 10.3748/wjg.v17.i39.4365]
50 **Ohara H**, Hoshino M, Hayakawa T, Kamiya Y, Miyaji M, Takeuchi T, Okayama Y, Gotoh K. Single application extracorporeal shock wave lithotripsy is the first choice for patients with pancreatic duct stones. *Am J Gastroenterol* 1996; **91**: 1388-1394 [PMID: 8678001]
51 **Dumonceau JM**, Costamagna G, Tringali A, Vahedi K, Delhaye M, Hittelet A, Spera G, Giostra E, Mutignani M, De Maertelaer V, Devière J. Treatment for painful calcified chronic pancreatitis: extracorporeal shock wave lithotripsy versus endoscopic treatment: a randomised controlled trial. *Gut* 2007; **56**: 545-552 [PMID: 17047101 DOI: 10.1136/gut.2006.096883]
52 **Delhaye M**, Arvanitakis M, Verset G, Cremer M, Devière J. Long-term clinical outcome after endoscopic pancreatic ductal drainage for patients with painful chronic pancreatitis. *Clin Gastroenterol Hepatol* 2004; **2**: 1096-1106 [PMID: 15625655 DOI: 10.1016/S1542-3565(04)00544-0]
53 **Seven G**, Schreiner MA, Ross AS, Lin OS, Gluck M, Gan SI, Irani S, Brandabur JJ, Patterson D, Kuhr C, Kozarek R. Long-term outcomes associated with pancreatic extracorporeal shock wave lithotripsy for chronic calcific pancreatitis. *Gastrointest Endosc* 2012; **75**: 997-1004.e1 [PMID: 22401819 DOI: 10.1016/j.gie.2012.01.014]
54 **Tandan M**, Reddy DN, Talukdar R, Vinod K, Santosh D, Lakhtakia S, Gupta R, Ramchandani MJ, Banerjee R, Rakesh K, Varadaraj G, Rao GV. Long-term clinical outcomes of extracorporeal shockwave lithotripsy in painful chronic calcific pancreatitis. *Gastrointest Endosc* 2013 Jul 25; Epub ahead of print [PMID: 23891416]
55 **Hirai T**, Goto H, Hirooka Y, Itoh A, Hashimoto S, Niwa Y, Hayakawa T. Pilot study of pancreatoscopic lithotripsy using a 5-fr instrument: selected patients may benefit. *Endoscopy* 2004; **36**: 212-216 [PMID: 14986218 DOI: 10.1055/s-2004-814250]
56 **Howell DA**, Dy RM, Hanson BL, Nezhad SF, Broaddus SB. Endoscopic treatment of pancreatic duct stones using a 10F pancreatoscope and electrohydraulic lithotripsy. *Gastrointest Endosc* 1999; **50**: 829-833 [PMID: 10570346 DOI: 10.1016/S0016-5107(99)70168-9]
57 **Barthet M**, Bernard JP, Duval JL, Affriat C, Sahel J. Biliary stenting in benign biliary stenosis complicating chronic calcifying pancreatitis. *Endoscopy* 1994; **26**: 569-572 [PMID: 8001482 DOI: 10.1055/s-2007-1009041]
58 **Kahl S**, Zimmermann S, Genz I, Glasbrenner B, Pross M, Schulz HU, Mc Namara D, Schmidt U, Malfertheiner P. Risk factors for failure of endoscopic stenting of biliary strictures in chronic pancreatitis: a prospective follow-up study. *Am J Gastroenterol* 2003; **98**: 2448-2453 [PMID: 14638347 DOI: 10.1111/j.1572-0241.2003.08667.x]
59 **Catalano MF**, Linder JD, George S, Alcocer E, Geenen JE. Treatment of symptomatic distal common bile duct stenosis secondary to chronic pancreatitis: comparison of single vs. multiple simultaneous stents. *Gastrointest Endosc* 2004; **60**: 945-952 [PMID: 15605010 DOI: 10.1016/S0016-5107(04)02275-8]
60 **Cahen DL**, Rauws EA, Gouma DJ, Fockens P, Bruno MJ. Removable fully covered self-expandable metal stents in the treatment of common bile duct strictures due to chronic pancreatitis: a case series. *Endoscopy* 2008; **40**: 697-700 [PMID: 18704837 DOI: 10.1055/s-2008-1077353]
61 **Behm B**, Brock A, Clarke BW, Ellen K, Northup PG, Dumonceau JM, Kahaleh M. Partially covered self-expandable metallic stents for benign biliary strictures due to chronic pancreatitis. *Endoscopy* 2009; **41**: 547-551 [PMID: 19533560 DOI: 10.1055/s-0029-1214708]
62 **Deviere JM**, Reddy DN, Puspok A, Ponchon T, Bruno MJ, Bourke MJ, Neuhaus H, Roy A, González-Huix F, Barkun AN, Kortan PP, Navarrete C, Costamagna G. 147 Preliminary Results From a 187 Patient Multicenter Prospective Trial Using Metal Stents for Treatment of Benign Biliary Strictures. *Gastrointest Endosc* 2012; **75**: AB123 [DOI: 10.1016/j.gie.2012.04.018]
63 **Telford JJ**, Farrell JJ, Saltzman JR, Shields SJ, Banks PA, Lichtenstein DR, Johannes RS, Kelsey PB, Carr-Locke DL. Pancreatic stent placement for duct disruption. *Gastrointest Endosc* 2002; **56**: 18-24 [PMID: 12085030 DOI: 10.1067/mge.2002.125107]
64 **Andrén-Sandberg A**, Dervenis C. Pancreatic pseudocysts in the 21st century. Part I: classification, pathophysiology, anatomic considerations and treatment. *JOP* 2004; **5**: 8-24 [PMID: 14730118]
65 **Lerch MM**, Stier A, Wahnschaffe U, Mayerle J. Pancreatic pseudocysts: observation, endoscopic drainage, or resection? *Dtsch Arztebl Int* 2009; **106**: 614-621 [PMID: 19890418]
66 **Andrén-Sandberg A**, Dervenis C. Pancreatic pseudocysts in the 21st century. Part II: natural history. *JOP* 2004; **5**: 64-70 [PMID: 15007187]
67 **Beckingham IJ**, Krige JE, Bornman PC, Terblanche J. Endoscopic management of pancreatic pseudocysts. *Br J Surg* 1997; **84**: 1638-1645 [PMID: 9448608 DOI: 10.1002/bjs.1800841204]
68 **Cahen D**, Rauws E, Fockens P, Weverling G, Huibregtse K, Bruno M. Endoscopic drainage of pancreatic pseudocysts: long-term outcome and procedural factors associated with safe and successful treatment. *Endoscopy* 2005; **37**: 977-983

[PMID: 16189770 DOI: 10.1055/s-2005-870336]
69 **Balachandra S**, Siriwardena AK. Systematic appraisal of the management of the major vascular complications of pancreatitis. *Am J Surg* 2005; **190**: 489-495 [PMID: 16105542 DOI: 10.1016/j.amjsurg.2005.03.009]
70 **Varadarajulu S**, Trevino J. Wilcox CM, Sutton B, Christein JD. Randomized trial comparing EUS and surgery for pancreatic pseudocyst drainage. *Gastrointest Endosc* 2010; **71**: AB116-AB116 [DOI: 10.1016/j.gie.2010.03.047]
71 **Hookey LC**, Debroux S, Delhaye M, Arvanitakis M, Le Moine O, Devière J. Endoscopic drainage of pancreatic-fluid collections in 116 patients: a comparison of etiologies, drainage techniques, and outcomes. *Gastrointest Endosc* 2006; **63**: 635-643 [PMID: 16564865 DOI: 10.1016/j.gie.2005.06.028]
72 **Banerjee S**, Shen B, Baron TH, Nelson DB, Anderson MA, Cash BD, Dominitz JA, Gan SI, Harrison ME, Ikenberry SO, Jagannath SB, Lichtenstein D, Fanelli RD, Lee K, van Guilder T, Stewart LE. Antibiotic prophylaxis for GI endoscopy. *Gastrointest Endosc* 2008; **67**: 791-798 [PMID: 18374919 DOI: 10.1016/j.gie.2008.02.068]
73 **Sanchez Cortes E**, Maalak A, Le Moine O, Baize M, Delhaye M, Matos C, Devière J. Endoscopic cystenterostomy of nonbulging pancreatic fluid collections. *Gastrointest Endosc* 2002; **56**: 380-386 [PMID: 12196776 DOI: 10.1016/S0016-5107(02)70042-4]
74 **Kahaleh M**, Shami VM, Conaway MR, Tokar J, Rockoff T, De La Rue SA, de Lange E, Bassignani M, Gay S, Adams RB, Yeaton P. Endoscopic ultrasound drainage of pancreatic pseudocyst: a prospective comparison with conventional endoscopic drainage. *Endoscopy* 2006; **38**: 355-359 [PMID: 16680634 DOI: 10.1055/s-2006-925249]
75 **Varadarajulu S**, Bang JY, Sutton BS, Trevino JM, Christein JD, Wilcox CM. Equal efficacy of endoscopic and surgical cystogastrostomy for pancreatic pseudocyst drainage in a randomized trial. *Gastroenterology* 2013; **145**: 583-590.e1 [PMID: 23732774]
76 **Kahaleh M**, Hernandez AJ, Tokar J, Adams RB, Shami VM, Yeaton P. EUS-guided pancreaticogastrostomy: analysis of its efficacy to drain inaccessible pancreatic ducts. *Gastrointest Endosc* 2007; **65**: 224-230 [PMID: 17141775 DOI: 10.1016/j.gie.2006.05.008]
77 **Tessier G**, Bories E, Arvanitakis M, Hittelet A, Pesenti C, Le Moine O, Giovannini M, Devière J. EUS-guided pancreatogastrostomy and pancreatobulbostomy for the treatment of pain in patients with pancreatic ductal dilatation inaccessible for transpapillary endoscopic therapy. *Gastrointest Endosc* 2007; **65**: 233-241 [PMID: 17258981 DOI: 10.1016/j.gie.2006.06.029]
78 **Brauer BC**, Chen YK, Fukami N, Shah RJ. Single-operator EUS-guided cholangiopancreatography for difficult pancreaticobiliary access (with video). *Gastrointest Endosc* 2009; **70**: 471-479 [PMID: 19560768 DOI: 10.1016/j.gie.2008.12.233]
79 **Puli SR**, Reddy JB, Bechtold ML, Antillon MR, Brugge WR. EUS-guided celiac plexus neurolysis for pain due to chronic pancreatitis or pancreatic cancer pain: a meta-analysis and systematic review. *Dig Dis Sci* 2009; **54**: 2330-2337 [PMID: 19137428 DOI: 10.1007/s10620-008-0651-x]
80 **Kaufman M**, Singh G, Das S, Concha-Parra R, Erber J, Micames C, Gress F. Efficacy of endoscopic ultrasound-guided celiac plexus block and celiac plexus neurolysis for managing abdominal pain associated with chronic pancreatitis and pancreatic cancer. *J Clin Gastroenterol* 2010; **44**: 127-134 [PMID: 19826273 DOI: 10.1097/MCG.0b013e3181bb854d]
81 **Gress F**, Schmitt C, Sherman S, Ciaccia D, Ikenberry S, Lehman G. Endoscopic ultrasound-guided celiac plexus block for managing abdominal pain associated with chronic pancreatitis: a prospective single center experience. *Am J Gastroenterol* 2001; **96**: 409-416 [PMID: 11232683 DOI: 10.1111/j.1572-0241.2001.03551.x]
82 **Santosh D**, Lakhtakia S, Gupta R, Reddy DN, Rao GV, Tandan M, Ramchandani M, Guda NM. Clinical trial: a randomized trial comparing fluoroscopy guided percutaneous technique vs. endoscopic ultrasound guided technique of coeliac plexus block for treatment of pain in chronic pancreatitis. *Aliment Pharmacol Ther* 2009; **29**: 979-984 [PMID: 19222416 DOI: 10.1111/j.1365-2036.2009.03963.x]

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MINIREVIEWS

HER2 therapies and gastric cancer: A step forward

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Abstract

Gastric cancer usually is diagnosed in advanced stages and thus current medical practice affords limited therapeutic options. However, recent studies established the role of human epidermal growth factor receptor 2 (HER2) in clinical management. Trastuzumab, an anti-HER2 monoclonal antibody, acquired a main role in advanced gastric cancer harboring HER2 overexpression and/or amplification improving survival to 17.1 mo according to trastuzumab for gastric cancer phase Ⅲ trial results. Also, new promising drugs, such as c-Met inhibitors, are in development and assessment for this setting. Certainly, novel drugs will emerge in the next feel years for help oncologists improve clinical management of advanced gastric cancer providing higher survival and quality of life. In this mini-review we will discuss some issues in this regard and provide an actual overview of this setting.

Key words: Gastric cancer; Trastuzumab; c-Met; Human epidermal growth factor receptor 2; Lapatinib; Pertuzumab

Core tip: Approaches for treatment advanced gastric cancer are object of interesting debates toward scientific community worldwide over the last 20 years. Chemotherapy based on platinum and fluoropirimidine agents remained up to now the standard of care for those patients, otherwise triplet therapy either an anthracycline or taxane may be considered. Herein we provide an additional discussion regarding the role of biologic agents, such as trastuzumab and novel therapies for improve survival in this field.

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INTRODUCTION

Gastric cancer (GC) is one of the leading types of cancer worldwide. Although the trend in death rates[1] for GC is decreasing, this tumor continues to have a poor prognosis and few efficacious therapeutic options particularly in advanced stages. Since most of symptoms for this type of cancer are nonspecific and screening strategies in many countries are absence, GC is usually diagnosed in advanced stages. The predominant histological type of GC is adenocarcinoma (95% of tumors) and the main adenocarcinomas sub-types are intestinal, diffuse and mixed type. Recent studies showed the human cancer is the human epidermal growth factor receptor in advanced GC personalizing treatment[2-5]. Herein we will discuss issues concerning novel biologic agents for advanced gastric cancer, focusing in anti-human epidermal growth factor receptor 2 (HER2) therapies, such as trastuzumab, and promising novel agents.

HER2 AND GASTRIC CANCER

Treatment depends on the site and extent of the tumor[4,6,7]. Treatment objectives vary from through curative approaches, such as curative surgery, radiotherapy and perioperative chemotherapy, that may improve the survival rate of operable GC patients; to palliative approaches in advanced stage patients or those who are subject to relapse after prior curative surgery[7,8]. For advanced patients, 5-fluorouracil (5-FU) plus platinum remain standard treatment regimens, with or without an anthracycline or taxane[9]. This therapeutic regimen offers a response rate of 30%-50% with 9-11 mo median overall survival (OS)[10]. Given these poor results, an investment in new treatment weapons is required. One of the most considerable innovative targets in human cancer is the human epidermal growth factor receptor (EGFR) family[11]. The human HER family includes four structurally related members, HER1 (ErbB1, also known as EGFR), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4)[12]. Relatively to HER2, this is highly expressed in a significant proportion of GC[13] and thus it is nowadays considered an excellent therapeutic target. GC harboring HER2 overexpression was shown to have a worse prognosis[14]. In HER2-amplified patients the median survival was 5.5 mo compared with 12.6 mo in non-amplified patients. HER2 overexpression was more commonly seen in the intestinal-type than diffuse-type cancers (32% *vs* 6%)[15-17].

HER2 MOLECULAR TESTS AND TRASTUZUMAB

HER2 overexpression can be determined by immunohistochemistry (IHC) using a monoclonal antibody or by the detection of HER2 gene amplification through fluorescent *in situ* hybridization (FISH)[18-20]. Thus, it is current practice to test all new diagnoses of GC for HER2 by IHC[21,22]. Tumors can be classified by IHC as IHC 0/1+, negative resulted; IHC2+, equivocal resulted and it is recommended FISH testing, and IHC3+, positive resulted[18,23].

In the trastuzumab for gastric cancer (ToGA) trial[2], trastuzumab, a recombinant humanized monoclonal antibody that targets the extracellular domain Ⅳ of the HER2 protein, was evaluated in HER2 overexpressing gastric and gastroesophageal junction (GEJC) cancer. In the mentioned study, patients with GC or GEJ that showed HER2 overexpression were eligible for the analysis and randomized in two arms. To one arm standard chemotherapy alone (5-FU/capecitabine plus cisplatin) was administered while to the other arm it was administered chemotherapy plus transtuzumab. Median OS was 13.8 mo in those assigned to trastuzumab plus chemotherapy compared with 11.1 mo in those assigned to chemotherapy alone[24]. The median of progression-free survival (PFS) was increased with the addition of transtuzumab to standard chemotherapy: 6.7 mo in the transtuzumab arm and 5.5 mo in the chemotherapy alone arm. The overall response rate was 47.3% *vs* 34.5% in transtuzumab plus chemotherapy and chemotherapy, respectively. The toxicity did not increased substantially with transtuzumab addition; however, the most common grade 3/4 adverse reactions associated with trastuzumab in metastatic GC were neutropenia, diarrhea, fatigue, anemia, stomatitis, weight loss, upper respiratory tract infections, fever, thrombocytopenia, mucosal inflammation, nasopharyngitis and dysgeusia. Thus, the ToGA trial showed that trastuzumab in combination with chemotherapy can be considered as a new standard option for patients with HER2-positive advanced GC or GEJC. So, trastuzumab was approved by the Food and Drug Administration and the European Medicines Agency (EMA) for patients with HER2-positive metastatic GC or GEJ who have not received previous anticancer therapy for metastatic disease.

NOVEL AGENTS AND PROMISING MOLECULES

Nevertheless, others monoclonal antibodies have been developed as an alternative to transtuzumab[25-28]. For example, HER dimerization inhibitor, such as pertuzumab, which in combination with the transtuzumab has shown to have a promising effect in experimental models of GC[29,30]. In addition, some studies with anti-HER2 combination treatments indicate that the use of more than one HER2-targeted therapy was superior to one of these agents alone, particularly in breast cancer (BC) HER2 positive[31-33]. For instance, the CLEOPATRA[34] phase Ⅲ trial compared the efficacy and safety of pertuzumab, trastuzumab, and docetaxel with placebo, trastuzumab, and docetaxel in patients with HER2-positive first-line metastatic breast cancer, showed a significant improvement in OS with addition of pertuzumab. So, there is need for planning studies to assess the safety and efficacy of the pertuzumab in the GC HER2 positive[35,36].

However, when the patients acquire resistance to transtuzumab, what to do? The molecular mechanisms underlying trastuzumab resistance in GC are still unknown, but intra-tumoral heterogeneity of this tumor may contribute to this resistance[12,37-39]. There are some mechanist theories in a study that attempted to explain this phenomenon, *e.g.*, that catecholamine-induced β2-AR activation mediates desensitization of GC cells to trastuzumab through up regulation of the MUC4 expression[40,41]; or that interaction between HER2 and insulin-like growth factor 1 receptor in trastuzumab-resistant breast cancer cells and involved in cross-talk that results in p27 downregulation[42]. Furthermore, hepatocyte growth factor (HGF) and its receptor, the trans-membrane tyrosine kinase c-Met, promote cell proliferation, survival, motility and invasion as well as morphologic changes that stimulate tissue repair and regeneration in normal cells but can be co-opted during tumor growth[28]. Previous studies reported that high levels of HGF or c-Met are associated with poor prognosis in gastric can-

cer, due to gene amplification and protein overexpression of c-Met drive resistance to epidermal growth factor receptor family inhibitors, both in preclinical models and in patients[21,27,28,43,44]. Only a few phase I - Ⅱ trials[26,45] recently assessed the role of c-Met inhibitors, such as crizotinib[46] and foretinib[26], in gastric cancer setting. In a studied by Lennerz *et al*[46] two patients harboring MET amplification were treated with crizotinb and presented tumor shrinkage (-30% and -16%) and experienced progression after 3.7 and 3.5 mo. Shah *et al*[26] reported 67 advanced gastric cancer patients who were treated with foretinib irrespective of c-Met status. Best response was stable disease (SD) in 10 (23%) patients receiving intermittent dosing and 5 (20%) receiving daily dosing; SD duration was 1.9-7.2 mo (median 3.2 mo). Of 67 patients with tumor samples, 3 had MET amplification, one of whom had SD. Treatment-related toxicity occurred in 91% of patients[26]. Thus, the response to this dilemma is not to simple and current there are many options for explore in this regard.

In this regard, others classes of targeted drugs, including tyrosine kinase inhibitors, such as lapatinib[47] and dacomitinib[48], mammalian target of rapamycin pathway inhibitors, such as everolimus[49], have also been investigated. Lapatinib inhibits the catalytic activity of the EGFR and it is also a HER2 inhibitor; thus, it is a dual tyrosine kinase inhibitor of both EGFR and HER2. The SWOG S413 trial[47] analyzed lapatinib in the first line therapy in patients with advanced or metastatic GC showing 9% response rate (11% overall response rate) and a median OS of 4.8 mo. In summary, lapatinib as a single agent presents reduced responses, but in combination with other chemotherapeutic agents may have additional benefits. Dacomitinib[18] is a pan-HER inhibitor with potential use in cancer treatment via mutations or overexpression/amplification of HER family members or their target molecules alone or in combination with chemotherapeutic and/or molecular-targeted agents, however, there are no clinical trials phase Ⅱ/Ⅲ to justify its use in GC patients.

CONCLUSION

Nowadays, an interesting biologic option is available, such as trastuzumab, for combination with platinum-5-FU for prolongs OS in a sub-set of patients. However, only 20% of advanced GC harbor with HER2 overexpression and thus a large number of patients will not acquire benefit from this innovative option. Thus, further alternatives are warranted for overcome this issue. Others biological agents are under investigation, but without immediate results for the current clinical practice. Crizotinib, foretinib and pertuzumab seems to be promising due to preliminaries small studies. However, results from larges phase Ⅲ trials are still need to determine whether those innovative agents would be place in the current scenario. In conclusion, HER2 targeted therapy is responsible for a significant increase in survival of patients with GC in advanced stages. Unfortunately, the GC continues to still have a poor prognosis. In the future it is intended to develop new trials and look for other genetic alterations that may be highly specific therapeutic targets and less toxic as well.

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REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]
2 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]
3 **Stoss O**, Nagelmeier I, Zielinski D, Rüschoff J. The ToGA (Trastuzumab for GAstric Cancer) Trial: Importance from a Biomarker Perspective. *Connection* 2010; **2010:** 52-53
4 **Bang Y**, Chung H, Xu J, Lordick F, Sawaki A, Lipatov O, Al-Sakaff N, See C, Rueschoff J, Cutsem EV. Pathological features of advanced gastric cancer (GC): Relationship to human epidermal growth factor receptor 2 (HER2) positivity in the global screening programme of the ToGA trial. *J Clin Oncol* 2009; **27**: 4556
5 **Smith I**, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J, Kaufmann M, Cameron D, Bell R, Bergh J, Coleman R, Wardley A, Harbeck N, Lopez RI, Mallmann P, Gelmon K, Wilcken N, Wist E, Sánchez Rovira P, Piccart-Gebhart MJ. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* 2007; **369**: 29-36 [PMID: 17208639 DOI: 10.1016/S0140-6736(07)60028-2]
6 **Ananiev J**, Gulubova M, Manolova I, Tchernev G. Prognostic significance of HER2/neu expression in gastric cancer. *Wien Klin Wochenschr* 2011; **123**: 450-454 [PMID: 21739203 DOI: 10.1007/s00508-011-0025-9]
7 **Croxtall JD**, McKeage K. Trastuzumab: in HER2-positive metastatic gastric cancer. *Drugs* 2010; **70**: 2259-2267 [PMID: 21080742 DOI: 10.2165/11205900-000000000-00000]
8 **de Mello RA**, Gregório T, Cardoso T. Chylous ascites due to signet ring cell gastric adenocarcinoma. *Niger J Clin Pract* 2012; **15**: 487-490 [PMID: 23238204 DOI: 10.4103/1119-3077.104536]
9 **Wagner AD**, Unverzagt S, Grothe W, Kleber G, Grothey A, Haerting J, Fleig WE. Chemotherapy for advanced gastric cancer. *Cochrane Database Syst Rev* 2010; (**3**): CD004064 [PMID: 20238327]
10 **De Vita F**, Giuliani F, Silvestris N, Catalano G, Ciardiello F, Orditura M. Human epidermal growth factor receptor 2 (HER2) in gastric cancer: a new therapeutic target. *Cancer Treat Rev* 2010; **36** Suppl 3: S11-S15 [PMID: 21129604]
11 **Fornaro L**, Lucchesi M, Caparello C, Vasile E, Caponi S, Ginocchi L, Masi G, Falcone A. Anti-HER agents in gastric cancer: from bench to bedside. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 369-383 [PMID: 21647199 DOI: 10.1038/nrgas-

tro.2011.81]
12 **Grabsch H**, Sivakumar S, Gray S, Gabbert HE, Müller W. HER2 expression in gastric cancer: Rare, heterogeneous and of no prognostic value - conclusions from 924 cases of two independent series. *Cell Oncol* 2010; **32**: 57-65 [PMID: 20208134 DOI: 10.3233/CLO-2009-0497]
13 **Tai W**, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. *J Control Release* 2010; **146**: 264-275 [PMID: 20385184 DOI: 10.1016/j.jconrel.2010.04.009]
14 **Song Y**, Huang J, Wang JW. [Relationship between HER2/neu gene amplification and protein expression and prognosis in patients with advanced gastric carcinoma]. *Chin J Cancer* 2010; **29**: 76-81 [PMID: 20038314]
15 **Bang YJ**, Chung HC, Xu JM, Lordick F, Sawaki A, Lipatov O, Al-Sakaff N, See CG, Rueschoff J, Van Cutsem E. Pathological features of advanced gastric cancer: relationship to human epidermal growth factor receptor 2 positivity in the global screening programme of the ToGA trial. *J Clin Oncol* 2009; **27**: 18s
16 **Gravalos C**, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol* 2008; **19**: 1523-1529 [PMID: 18441328 DOI: 10.1093/annonc/mdn169]
17 **Janjigian YY**, Werner D, Pauligk C, Steinmetz K, Kelsen DP, Jäger E, Altmannsberger HM, Robinson E, Tafe LJ, Tang LH, Shah MA, Al-Batran SE. Prognosis of metastatic gastric and gastroesophageal junction cancer by HER2 status: a European and USA International collaborative analysis. *Ann Oncol* 2012; **23**: 2656-2662 [PMID: 22689179 DOI: 10.1093/annonc/mds104]
18 **Hofmann M**, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008; **52**: 797-805 [PMID: 18422971 DOI: 10.1111/j.1365-2559.2008.03028.x]
19 **Hede K**. Gastric cancer: trastuzumab trial results spur search for other targets. *J Natl Cancer Inst* 2009; **101**: 1306-1307 [PMID: 19755679 DOI: 10.1093/jnci/djp341]
20 **Nakajima M**, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, Matsuda M, Sakaguchi T, Hirao T, Nakano H. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer* 1999; **85**: 1894-1902 [PMID: 10223227]
21 **Okines AF**, Thompson LC, Cunningham D, Wotherspoon A, Reis-Filho JS, Langley RE, Waddell TS, Noor D, Eltahir Z, Wong R, Stenning S. Effect of HER2 on prognosis and benefit from peri-operative chemotherapy in early oesophago-gastric adenocarcinoma in the MAGIC trial. *Ann Oncol* 2013; **24**: 1253-1261 [PMID: 23233651 DOI: 10.1093/annonc/mds622]
22 **Pazo Cid RA**, Antón A. Advanced HER2-positive gastric cancer: current and future targeted therapies. *Crit Rev Oncol Hematol* 2013; **85**: 350-362 [PMID: 23021388 DOI: 10.1016/j.critrevonc.2012.08.008]
23 **Rüschoff J**, Dietel M, Baretton G, Arbogast S, Walch A, Monges G, Chenard MP, Penault-Llorca F, Nagelmeier I, Schlake W, Höfler H, Kreipe HH. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch* 2010; **457**: 299-307 [PMID: 20665045 DOI: 10.1007/s00428-010-0952-2]
24 **Gunturu KS**, Woo Y, Beaubier N, Remotti HE, Saif MW. Gastric cancer and trastuzumab: first biologic therapy in gastric cancer. *Ther Adv Med Oncol* 2013; **5**: 143-151 [PMID: 23450234 DOI: 10.1177/1758834012469429]
25 **Pishvaian M**, Sakaeva D, Hsieh R. A global, multi-center phase II trial of lapatinib plus capecitabine in gastric cancer. *J Clin Oncol* 2011; **29** Suppl 4: abstr 88
26 **Shah MA**, Wainberg ZA, Catenacci DV, Hochster HS, Ford J, Kunz P, Lee FC, Kallender H, Cecchi F, Rabe DC, Keer H, Martin AM, Liu Y, Gagnon R, Bonate P, Liu L, Gilmer T, Bottaro DP. Phase II study evaluating 2 dosing schedules of oral foretinib (GSK1363089), cMET/VEGFR2 inhibitor, in patients with metastatic gastric cancer. *PLoS One* 2013; **8**: e54014 [PMID: 23516391 DOI: 10.1371/journal.pone.0054014]
27 **Blumenschein GR**, Mills GB, Gonzalez-Angulo AM. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. *J Clin Oncol* 2012; **30**: 3287-3296 [PMID: 22869872 DOI: 10.1200/JCO.2011.40.3774]
28 **Barok M**, Tanner M, Köninki K, Isola J. Trastuzumab-DM1 is highly effective in preclinical models of HER2-positive gastric cancer. *Cancer Lett* 2011; **306**: 171-179 [PMID: 21458915 DOI: 10.1016/j.canlet.2011.03.002]
29 **Yamashita-Kashima Y**, Iijima S, Yorozu K, Furugaki K, Kurasawa M, Ohta M, Fujimoto-Ouchi K. Pertuzumab in combination with trastuzumab shows significantly enhanced antitumor activity in HER2-positive human gastric cancer xenograft models. *Clin Cancer Res* 2011; **17**: 5060-5070 [PMID: 21700765 DOI: 10.1158/1078-0432.ccr-10-2927]
30 **Sendur MA**, Aksoy S, Altundag K. Pertuzumab in HER2-positive breast cancer. *Curr Med Res Opin* 2012; **28**: 1709-1716 [PMID: 22953713]
31 **Baselga J**, Swain SM. CLEOPATRA: a phase III evaluation of pertuzumab and trastuzumab for HER2-positive metastatic breast cancer. *Clin Breast Cancer* 2010; **10**: 489-491 [PMID: 21147694 DOI: 10.3816/CBC.2010.n.065]
32 **Baselga J**, Cortés J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A, Clark E, Benyunes MC, Ross G, Swain SM. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* 2012; **366**: 109-119 [PMID: 22149875 DOI: 10.1056/NEJMoa1113216]
33 **Gianni L**, Lladó A, Bianchi G, Cortes J, Kellokumpu-Lehtinen PL, Cameron DA, Miles D, Salvagni S, Wardley A, Goeminne JC, Hersberger V, Baselga J. Open-label, phase II, multicenter, randomized study of the efficacy and safety of two dose levels of Pertuzumab, a human epidermal growth factor receptor 2 dimerization inhibitor, in patients with human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol* 2010; **28**: 1131-1137 [PMID: 20124183 DOI: 10.1200/JCO.2009.24.1661]
34 **Swain SM**, Kim SB, Cortés J, Ro J, Semiglazov V, Campone M, Ciruelos E, Ferrero JM, Schneeweiss A, Knott A, Clark E, Ross G, Benyunes MC, Baselga J. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol* 2013; **14**: 461-471 [PMID: 23602601 DOI: 10.1016/S1470-2045(13)70130-X]
35 **Boku N**. HER2-positive gastric cancer. *Gastric Cancer* 2013; Epub ahead of print [PMID: 23563986 DOI: 10.1007/s10120-013-0252-z]
36 **de Mello RA**, de Vasconcelos A, Ribeiro RA, Pousa I, Afonso N, Pereira D, Rodrigues H. Insight into p95HER2 in breast cancer: molecular mechanisms and targeted therapies. *Recent Pat DNA Gene Seq* 2012; **6**: 56-63 [PMID: 22239685]
37 **Barros-Silva JD**, Leitão D, Afonso L, Vieira J, Dinis-Ribeiro M, Fragoso M, Bento MJ, Santos L, Ferreira P, Rêgo S, Brandão C, Carneiro F, Lopes C, Schmitt F, Teixeira MR. Association of ERBB2 gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients. *Br J Cancer* 2009; **100**: 487-493 [PMID: 19156142 DOI: 10.1038/sj.bjc.6604885]
38 **Bystricky B**, Okines A, Cunningham D. Targeting Her-2 in gastric cancer - incorporation of trastuzumab into the treatment of operable disease. *Gastrointestinal Cancer: Targets and Therapy* 2011; **1**: 41-52 [DOI: 10.2147/GICTT.S18495]
39 **Gong SJ**, Jin CJ, Rha SY, Chung HC. Growth inhibitory effects of trastuzumab and chemotherapeutic drugs in gastric cancer cell lines. *Cancer Lett* 2004; **214**: 215-224 [PMID: 15363548 DOI: 10.1016/j.canlet.2004.04.029]
40 **Uchino S**, Tsuda H, Maruyama K, Kinoshita T, Sasako M, Saito T, Kobayashi M, Hirohashi S. Overexpression of

c-erbB-2 protein in gastric cancer. Its correlation with long-term survival of patients. *Cancer* 1993; **72**: 3179-3184 [PMID: 7902202]

41 **Fessler SP**, Wotkowicz MT, Mahanta SK, Bamdad C. MUC1* is a determinant of trastuzumab (Herceptin) resistance in breast cancer cells. *Breast Cancer Res Treat* 2009; **118**: 113-124 [PMID: 19415485 DOI: 10.1007/s10549-009-0412-3]

42 **Nahta R**, Yuan LX, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res* 2005; **65**: 11118-11128 [PMID: 16322262 DOI: 10.1158/0008-5472.can-04-3841]

43 **Kaji M**, Yonemura Y, Harada S, Liu X, Terada I, Yamamoto H. Participation of c-met in the progression of human gastric cancers: anti-c-met oligonucleotides inhibit proliferation or invasiveness of gastric cancer cells. *Cancer Gene Ther* 1996; **3**: 393-404 [PMID: 8988842]

44 **Kubicka S**, Claas C, Staab S, Kühnel F, Zender L, Trautwein C, Wagner S, Rudolph KL, Manns M. p53 mutation pattern and expression of c-erbB2 and c-met in gastric cancer: relation to histological subtypes, Helicobacter pylori infection, and prognosis. *Dig Dis Sci* 2002; **47**: 114-121 [PMID: 11837710]

45 **Yap TA**, Olmos D, Brunetto AT, Tunariu N, Barriuso J, Riisnaes R, Pope L, Clark J, Futreal A, Germuska M, Collins D, deSouza NM, Leach MO, Savage RE, Waghorne C, Chai F, Garmey E, Schwartz B, Kaye SB, de Bono JS. Phase I trial of a selective c-MET inhibitor ARQ 197 incorporating proof of mechanism pharmacodynamic studies. *J Clin Oncol* 2011; **29**: 1271-1279 [PMID: 21383285 DOI: 10.1200/JCO.2010.31.0367]

46 **Lennerz JK**, Kwak EL, Ackerman A, Michael M, Fox SB, Bergethon K, Lauwers GY, Christensen JG, Wilner KD, Haber DA, Salgia R, Bang YJ, Clark JW, Solomon BJ, Iafrate AJ. MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J Clin Oncol* 2011; **29**: 4803-4810 [PMID: 22042947 DOI: 10.1200/JCO.2011.35.4928]

47 **Iqbal S**, Goldman B, Fenoglio-Preiser CM, Lenz HJ, Zhang W, Danenberg KD, Shibata SI, Blanke CD. Southwest Oncology Group study S0413: a phase II trial of lapatinib (GW572016) as first-line therapy in patients with advanced or metastatic gastric cancer. *Ann Oncol* 2011; **22**: 2610-2615 [PMID: 21415234 DOI: 10.1093/annonc/mdr021]

48 **Nam HJ**, Ching KA, Kan J, Kim HP, Han SW, Im SA, Kim TY, Christensen JG, Oh DY, Bang YJ. Evaluation of the antitumor effects and mechanisms of PF00299804, a pan-HER inhibitor, alone or in combination with chemotherapy or targeted agents in gastric cancer. *Mol Cancer Ther* 2012; **11**: 439-451 [PMID: 22135232 DOI: 10.1158/1535-7163.mct-11-0494]

49 **Yoon DH**, Ryu MH, Park YS, Lee HJ, Lee C, Ryoo BY, Lee JL, Chang HM, Kim TW, Kang YK. Phase II study of everolimus with biomarker exploration in patients with advanced gastric cancer refractory to chemotherapy including fluoropyrimidine and platinum. *Br J Cancer* 2012; **106**: 1039-1044 [PMID: 22343617]

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ORIGINAL ARTICLE

Alteration in gene expression profile and oncogenicity of esophageal squamous cell carcinoma by *RIZ1* upregulation

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Abstract

AIM: To investigate the effect of retinoblastoma protein-interacting zinc finger gene 1 (*RIZ1*) upregulation in gene expression profile and oncogenicity of human esophageal squamous cell carcinoma (ESCC) cell line TE13.

METHODS: TE13 cells were transfected with pcDNA3.1(+)/RIZ1 and pcDNA3.1(+). Changes in gene expression profile were screened and the microarray results were confirmed by reverse transcription-polymerase chain reaction (RT-PCR). Nude mice were inoculated with TE13 cells to establish ESCC xenografts. After two weeks, the inoculated mice were randomly divided into three groups. Tumors were injected with normal saline, transfection reagent pcDNA3.1(+) and transfection reagent pcDNA3.1(+)/RIZ1, respectively. Tumor development was quantified, and changes in gene expression of RIZ1 transfected tumors were detected by RT-PCR and Western blotting.

RESULTS: DNA microarray data showed that RIZ1 transfection induced widespread changes in gene expression profile of cell line TE13, with 960 genes upregulated and 1163 downregulated. Treatment of tumor xenografts with RIZ1 recombinant plasmid significantly inhibited tumor growth, decreased tumor size, and increased expression of RIZ1 mRNA compared to control groups. The changes in gene expression profile were also observed *in vivo* after RIZ1 transfection. Most of the differentially expressed genes were associated with cell development, supervision of viral replication, lymphocyte costimulatory and immune system development in esophageal cells. *RIZ1* gene may be involved in multiple cancer pathways, such as cytokine receptor interaction and transforming growth factor beta signaling.

CONCLUSION: The development and progression of esophageal cancer are related to the inactivation of RIZ1. Virus infection may also be an important factor.

Key words: Retinoblastoma protein-interacting zinc finger gene 1; Microarray; Nude mice; Esophageal squamous cell carcinoma cells

Core tip: Retinoblastoma protein-interacting zinc finger gene 1 (*RIZ1*) transfection induced widespread changes in gene expression profile of cell line TE13, with 960 genes upregulated and 1163 downregulated. Most of the differentially expressed genes are associated with cell development, supervision of viral replication, lymphocyte costimulatory, and immune system development in esophageal cells. *RIZ1* gene may be involved in multiple cancer pathways, such as cytokine receptor interaction and transforming growth factor beta signaling. Virus infection may also be an important factor in

the development of esophageal cancer.

Dong SW, Li D, Xu C, Sun P, Wang YG, Zhang P. Alteration in gene expression profile and oncogenicity of esophageal squamous cell carcinoma by *RIZ1* upregulation. *World J Gastroenterol* 2013; 19(37): 6170-6177 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6170.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6170

INTRODUCTION

Esophageal cancer is one of the most common forms of cancer in the world and is a leading cause of cancer deaths in China and other developing countries. To date, the mechanisms of esophageal cancer are unclear. Tumor occurrence and development are regulated by a variety of oncogenes and tumor suppressor genes[1-3], including the putative tumor suppressor gene, Retinoblastoma protein-interacting zinc finger gene 1 (*RIZ1*). The *RIZ* gene has two expression products: RIZ1, which is believed to be a histone methyltransferase and acts on the locus of H3K9; and RIZ2, which lacks the PR-domain of RIZ1. Abnormal expression of RIZ1 has been found to be associated with tumor invasion and malignancy[4-10].

Our group has previously reported that RIZ1 expression level is lower in esophagus carcinoma than in adjacent noncancerous tissues[11], and is related to methylation of CpG islands[12]. In addition, by constructing human RIZ1 eukaryotic expression vectors to transfect human esophageal squamous cell carcinoma (ESCC) cell line TE13, we were able to report that upregulation of RIZ1 can recover tumor suppression activity and that treatment of cell line TE13 by methyltransferase inhibitor 5-aza-CdR reverses the methylation status of the promoter region[13]. In order to investigate RIZ1-mediated changes in gene expression of esophageal cancer, we compared the gene expression profile of TE13 cells transfected with RIZ1 with those of negative control cells. The resulting changes in oncogenicity were analyzed *in vitro* and by animal experimentation.

MATERIALS AND METHODS

Ethics

The animal study proposal was approved by the Tianjin Medical University General Hospital Ethics Committee with the permit number: 2012-021. All mouse experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China.

Cell culture and transfection

Human ESCC cell line TE13 was purchased from ATCC (Rcokville; MD, United States) and cultured in RPMI-1640 (HEPES 4.76 g/$NaCO_3$ 2.0 g/RPMI-1640 10.4 g/ddH_2O 1000 mL) media supplemented with 10% new-born bovine serum, 2 mmol/L 1 × *L*-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Life Technologies; NY, United States). Cells were maintained at 37 ℃ in a humidified atmosphere with 5% CO_2. RIZ1 eukaryotic expression vector pcDNA3.1(+)/RIZ1 plasmid had been prepared previously and stored at -80 ℃. Cultivated TE13 cells were passaged in 12-inch orifice plates. Passaging was repeated every 2-3 d at 1:10 dilution, and cells were lifted by trypsin digestion. When the cells were at the log phase, they were transfected using the classical liposome method by adding 2 μL Lipofectamine 2000 (Life Technologies; NY, United States). Experimental and control groups were transfected with pcDNA3.1(+)/RIZ1 and pcDNA3.1(+), respectively. The media were changed after 6 h and the cells were washed in phosphate buffered saline (PBS), harvested and counted. After mixing with Trizol Reagent (Life Technologies, NY, United States), the cells were incubated at room temperature for 5 min, then transferred to liquid nitrogen. The resultant cDNA was taken and 0.75 μL was mixed with 2 × SYBR Premix Ex TaqTM (Takara). The following primer sets (10 μmol/L) were used: RIZ1, forward 5'-TCTGCTGTTGACAAGACCC-3', reverse 5'-GCATCAATGCACATCCATC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5'-ACCCAGAAGACTGTGGATGG-3', reverse 5'-TTCAGCTCAGGGATGACCTT-3'. Amplification was carried out using LightCycler real-time polymerase chain reaction (PCR) system (Roche, United States), according to the manufacturer's protocol. Each sample was run in triplicate for each gene. An initial denaturation step at 95 ℃ for 5 min was followed by 40 denaturation cycles at 94 ℃ for 30 s, annealing at 56 ℃ for 30 s, and extension at 72 ℃ for 30 s. A solubility temperature curve assay was constructed and the RIZ1 and GAPDH Ct-values for each group were recorded and compared. The RIZ1 mRNA relative quantitation formula: ($2^{-\Delta\Delta Ct,}$ × 100%), was applied to evaluate whether transfection was successful. Duplicate detections were performed in triplicate, through $2^{-\Delta\Delta Ct}$, to calculate mean ± SD.

Microarray analysis

Total RNA was extracted using Trizol Reagent (Life Technologies; NY, United States). Quality control was achieved by utilizing the Agilent Bioanalyzer 2100 (Agilent Technologies; United States). Purification was achieved using the RNeasy mini kit and RNase-Free DNase Set (QIAGEN, Germany). Agilent's Low Input Quick Amp Labeling Kit, one-color and full genome chip (4 × 44K, design ID: 014850) were used to amplify and mark the mRNA according to the manufacturer's protocols. The cRNA was purified and conjugated using the RNeasy mini kit. Agilent's Gene Expression Hybridization Kit with a sample quantity of 1.65 μg cRNA was employed for gene chip hybridization for 17 h in a hybridization oven at < 65 ℃ and 10 rpm, following Agilent's protocol. Slides were washed in staining dishes (Thermo Shandon,

PA, United States) with Gene Expression Wash Buffer Kit. After completion of hybridization, gene chip scanning was performed using an Agilent Microarray Scanner. The software was adjusted to set the Dye channel to Green; scan resolution to 5 μm; and PMT to 100%, 10%, 16 bit. Data was read by Feature Extraction software v.10.7 and Gene Spring Software v.11.0, and uniformly processed by a Quantile algorithm. Data analysis was carried out using an online analysis system (SAS) (Shanghai Bohao Company, China). Fold changes ≥ 2 (upregulated) or ≤ 0.5 (downregulated) were set to select differentially expressed genes. Gene ontology (GO) functional analysis, chromosomal assignment, and pathway analysis were then performed. Several representative genes were selected for verification of the gene chip scan results by reverse transcription PCR (RT-PCR).

Animal experimentation

Purchase and feeding: Eighteen BALB/c nude mice (female; aged 4-6 wk; weight, 17-21 g), were purchased from the Experimental Animal Center of the Academy of Military Medical Sciences, Chinese People's Liberation Army, under license No. SCXK-(army)-2007-004. Nude mice were fed in the Tianjin Medical University General Hospital SPF rearing chamber with sterilized standard feed and sterile water. Animal experimentation and protocol were carried out in accordance with medical ethical standards.

Subcutaneous transplantation: Cultured cells were adjusted to a final concentration of 1×10^7 cells/mL in sterilized PBS; 0.2 mL was injected into the right armpit of each nude mouse and their general condition and tumor growth after inoculation were observed. The large and small tumors in diameter were measured every three days, tumor size was calculated and a tumor growth curve plotted. The antitumor rate was calculated using the following formulae: Volume = 0.5 × small diameter × (large diameter)^2; antitumor rate = (1 - the experimental group volume/the control group volume) × 100%.

Animal grouping and treatment: The nude mice were divided into three groups using a random digital method. Five mice from each group, with the maximum and minimum tumor volumes, were separated and the remaining mouse was removed. The blank control group was injected with 100 μL normal saline. The pcDNA3.1(+) group was injected with 20 μL liposome, to which 20 μg empty plasmid, adjusted to a final volume of 100 μL in DMEM, was added. The pcDNA3.1(+)/RIZ1 recombinant plasmid group was injected with 20 μL liposome to which 20 μg RIZ1 recombinant plasmid, adjusted to a final volume of 100 μL in DMEM, was added. Each group was injected every two days using the multi-point injection method, and the treatment was repeated 5 times. Seven days after the final injection, the mice were killed by cervical dislocation and the tumor tissue was removed, weighed and used for subsequent experimentation.

RT-PCR

RT-PCR was carried out as described above, and 12 mL of the reaction products were analyzed by electrophoresis on a 20 g/L agarose gel. The electrophoresis images were scanned by UV spectrophotometry (Beckman Coulter Inc., Brea, CA, United States).

Western blotting

Tissue from each group was homogenized in RIPA buffer (50 mmol/L Tris-HCl, pH 7.4; 150 mmol/L NaCl; 1% Nonidet P-40; 0.5% sodium deoxycholate; 0.1% SDS; 1 mmol/L EDTA; 1 mmol/L PMSF; 1 mg/mL Aprotinin). The supernatant was collected and protein concentrations were determined by a bicinchoninic acid (BCA) protein assay kit (Pierce; IL, United States); 30 μg of whole-cell lysate was separated on 8% SDS-PAGE gels, transferred to nitrocellulose (NC) membranes (Amersham Biosciences; New Jersey, United States), and immunoblotted with the following antibodies: anti-β-actin (ABCAM; United Kingdom), control; primary antibodies, 1:2000 dilution (ABCAM; United Kingdom); secondary antibodies Goat Anti-Mouse, 1:5000 dilution (ABCAM; United Kingdom). The films were analyzed by a Power-Look scanner (UMAX) and quantified by Image Quant software (GE; United States). The control experiments (TE13 cells; TE-13 cells transfected with blank plasmid) were treated by the same method. Relative expression of RIZ1 = gray value of RIZ1 protein/gray value of β-actin.

RESULTS

Transfection

The melting curve peaks for RIZ1 and GAPDH transcript products were at 80 ℃ and 82.5 ℃, respectively (Figure 1), giving Ct-values for the two groups. The relative expression levels were calculated using the real-time PCR relative quantitative formula. *RIZ1* gene expression levels were compared with SPSS v.13.0 statistical software. The results showed that the mRNA expression level in the experimental group was higher than in the control groups (Figure 2), indicating that transfection had been successful ($P \leq 0.01$).

Alteration of gene expression profile

Table 1 gives the 2100 results for RIN ≥ 7.0 and 28S/18S ≥ 0.7, therefore qualifying the samples without degradation. The initial scanned single fluorescence chip data (Figure 3A) were standardized and converted to logarithmic values. A scatter plot was constructed with a two-dimensional rectangular coordinate plane (Figure 3B).

GO and microarray analysis

The SAS system was used for GO analysis of the differentially expressed genes and $P \leq 0.05$ was considered to be statistically significant (Table 2). The microarray data showed that 2123 genes were differentially expressed in the pcDNA3.1(+)/RIZ1 transfected cells with fold

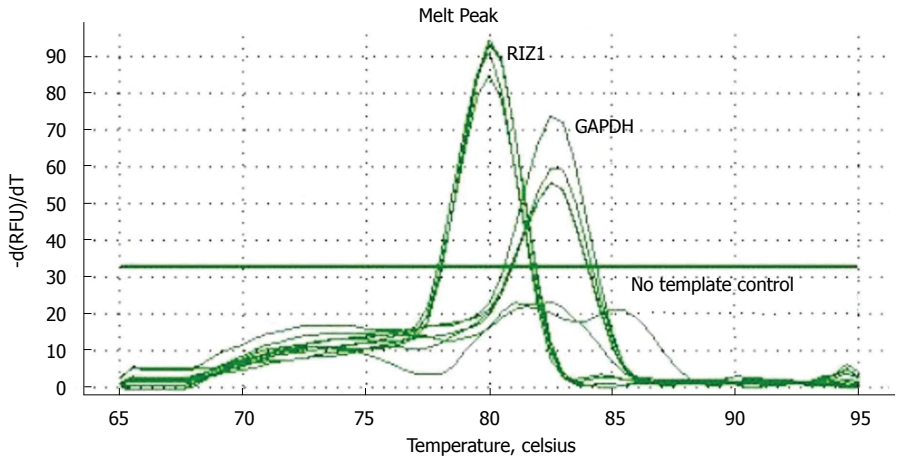

Figure 1 Solubility temperature curves for RIZ1 and glyceraldehyde 3-phosphate dehydrogenase showing that transfection was successful. DAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

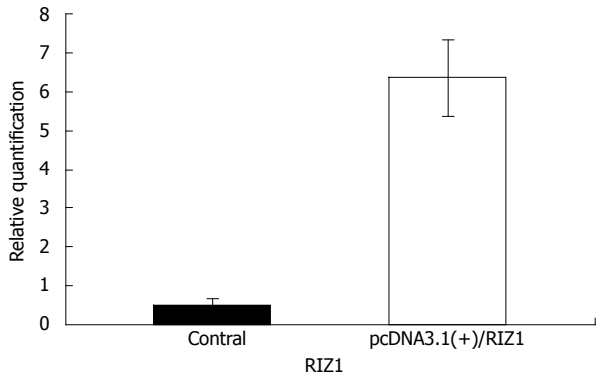

Figure 2 Histogram of *RIZ1* gene mRNA expression levels in the blank control group and pcDNA3.1(+)/RIZ1 recombinant plasmid group, clearly showing that gene expression is higher in the pcDNA3.1(+)/RIZ1 group ($P \leqslant 0.01$).

Table 1 Sample qualification

Sample number	Concentration (μg/μL)	A_{260}/A_{280}	2100 result		Results
			RIN	28S/18S	
1	0.816	1.94	8.9	1.5	Qualified
2	0.865	1.93	8.9	1.5	Qualified

changes > 2 ($P < 0.05$) compared to control samples. Of these, 960 genes were upregulated, of which 654 were known genes (1.70%, 654/38500) and 306 were unknown; 1163 genes were downregulated, of which 719 were known genes (1.87%, 719/38500) and 444 were unknown. Subsequent analyses were primarily carried out on annotated genes. The gene chip results were confirmed by RT-PCR (Figure 4).

Pathway analysis

Many of the identified genes are associated with cell development, virus replication supervision, costimulatory molecule, and immune system development. Further analysis indicated that the *RIZ1* gene may participate in multiple signaling pathways ($P < 0.01$), some of which are given in Table 3.

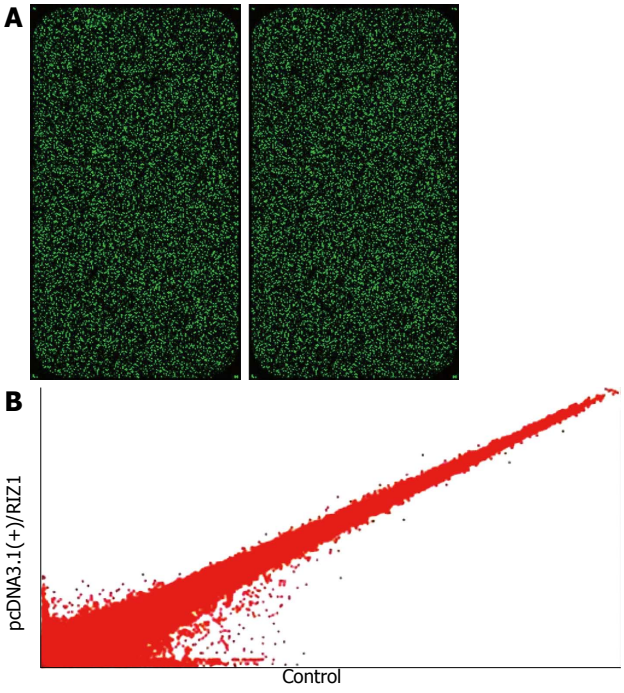

Figure 3 Alteration of gene expression profile. A: Single fluorescence chip images for microarray analysis; B: The data were used to construct the scatter plot. Hybridization signal strength scatter diagram. Each point in the scatter diagram represents a probe point on the chip. The position of each point is identified by an *x* and *y* coordinate: the *x*-coordinate gives the standardized signal value on the control chip; the *y*-coordinate gives the standardized signal value on the sample chip. The scatter plot is used to assess the centralized tendency of two sets of data.

Transplantation and tumor growth

Transplantation of human esophageal cancer TE13 cells into nude mice was successful as shown in Figure 5. Tumor volumes were compared among the three groups (Figure 6A). The tumor growth rate curves revealed that tumor growth was slower in the pcDNA3.1(+)/RIZ1 group, with a shallower growth rate curve, than in the

Table 2 Gene ontology analysis of differentially expressed genes

GO ID	Name	Hits	Total	Percentage	*P* value
GO: 0048468	Cell development	81	832	9.74%	0.0288
GO: 0050792	Regulation of viral reproduction	5	20	25.00%	0.0304
GO: 0031294	Lymphocyte costimulation	3	8	37.50%	0.0397
GO: 0002520	Immune system development	38	358	10.61%	0.0428

Hits: Number of differentially expressed genes in the pathway; Total: The total number of genes in the pathway; Percentage: Percentage hits in the pathway (Hits/Total); *P*: Enrichment *P* value; GO: Gene ontology.

Table 3 RIZ1 pathway analysis (selected pathways)

Name	Hits	Total	Percentage	*P* value	*q*
Cytokine-cytokine receptor interaction	25	276	9.06%	0	0
TGF-beta signaling pathway	10	85	11.76%	3.00E-04	5.00E-04
MAPK signaling pathway	20	271	7.38%	2.00E-04	5.00E-04
Pathways in cancer	20	328	6.10%	0.0018	0.0015

Hits: Number differentially expressed genes in the pathway; Total: Total number of genes in the pathway; Percentage: Percentage of hits in the pathway (Hits/Total); *P*: Enrichment *P* value; *q*: False discovery rate; TGF: Transforming growth factor; MAPK: Mitogen-activated protein kinase.

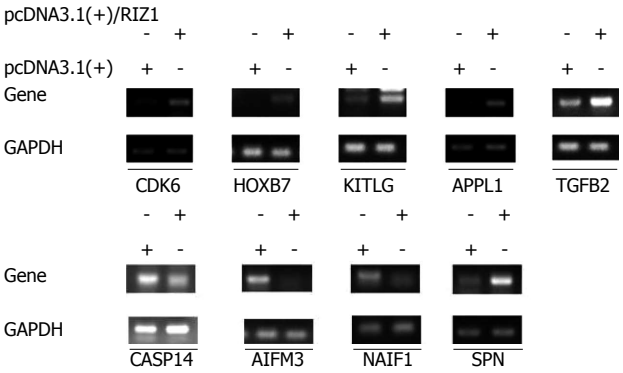

Figure 4 Comparison between results from the gene chip and the reverse transcription-polymerase chain reaction. Out of the nine genes tested, eight yielded consistent results for both reverse transcription-polymerase chain reaction and gene chip. The five upregulated genes are CDK6, HOXB7, KITLG, APPL1, TGFB2; and the three downregulated genes are CASP14, AIFM3 and NAIF1; however, in the case of service principal name (SPN), the reverse transcription-polymerase chain reaction results indicate upregulation, whereas the gene chip results indicate downregulation.

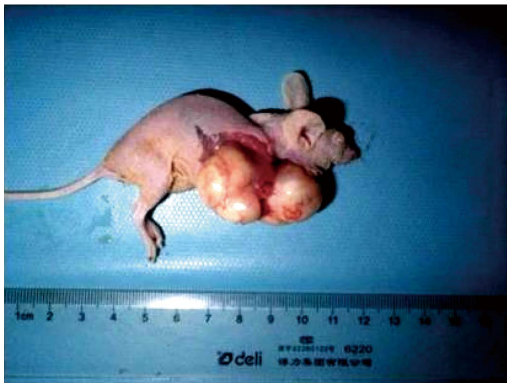

Figure 5 Photograph showing tumor development in a nude mouse, confirming successful transplantation of human esophageal cancer into an animal model.

other two groups (Figure 6B). The following tumor volumes were recorded after 26 d: 1.025 ± 0.018 cm^3 for the pcDNA3.1(+)/RIZ1 group, 2.208 ± 0.092 cm^3 for the blank control group, and 1.980 ± 0.089 cm^3 for the pcDNA3.1(+) group, showing that the tumor volume of the pcDNA3.1(+)/RIZ1 group was significantly lower than that of the other two groups ($P < 0.05$). In contrast, there was no significant difference between the blank control and pcDNA3.1(+) groups ($P > 0.05$).

Electrophoresis

RNA was extracted from the transplanted tumors for RT-PCR, and the products were analyzed by electrophoresis: GAPDH was identified at 125 base pairs (bp) and RIZ1 at 167 bp. The intensity of the GAPDH band was similar among the three groups; however, the intensity of the RIZ1 band was greatest in the pcDNA3.1(+)/RIZ1 group, showing that RIZ1 expression level was higher than in the other two groups (Figure 7).

Western blotting

The housekeeping gene, β-actin, was used as a control for Western blot analysis (Figure 8). The results showed no obvious difference in density of the β-actin bands among the three groups. In contrast, the density of the RIZ1 band was higher in the pcDNA3.1(+)/RIZ1 group than in the blank control and pcDNA3.1(+) groups, indicating that RIZ1 had been successfully expressed in the tumor tissues.

DISCUSSION

Esophageal cancer is the world's most common cancer of the digestive system, with 70% of incidences occurring in China. China also has the highest incidence and mortality rates for both men and women. Recent statistics on morbidity and mortality for cancer patients show that esophageal cancer is the 6th most common form of cancer, and the 4th highest cause of cancer death-related in China[14-18]. Furthermore, the incidence rate is higher in rural areas than in urban areas. Esophageal cancer can be divided into two pathological types: ESCC and esophageal adenoid carcinoma. In China, ESCC accounts for 90% of esophageal cancers, in contrast to Western countries.

Treatment of esophageal carcinoma is a long-term project; however, by combining several different treatment types, the quality of life of the patients can be greatly improved. The purpose of our study is to enhance the understanding of ESCC development and mechanisms at the genetic level in order to advance clinical therapies. RIZ1 is one of the most effective tumor sup-

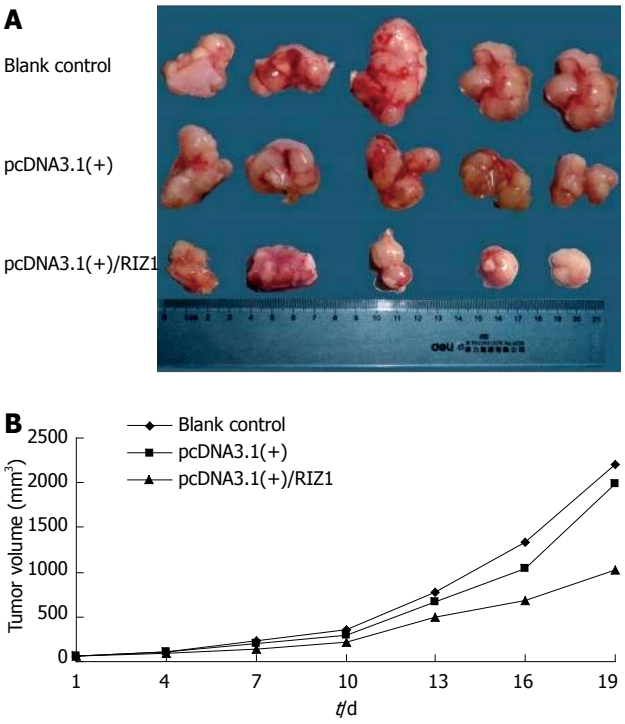

Figure 6 Transplantation and tumor growth. A: Photograph comparing esophageal squamous cell carcinomas removed from nude mice of the three groups after 26 d; B: Growth rate curves showing the increase in tumor volumes with time. The shallower curve in the pcDNA3.1(+)/RIZ1 group, compared to the blank control and pcDNA3.1(+) groups, indicates slower growth.

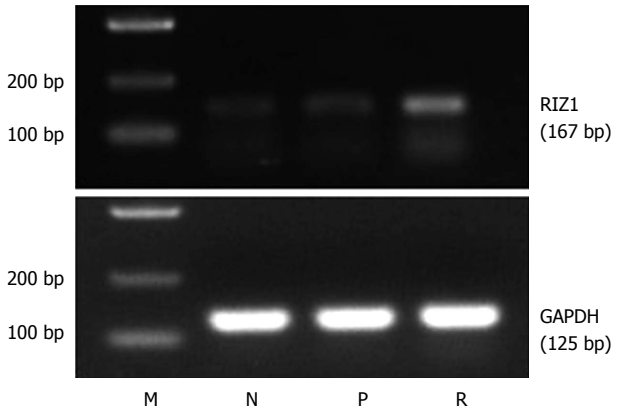

Figure 7 Electrophoresis gel image showing bands for glyceraldehyde 3-phosphate dehydrogenase (125 bp) and RIZI (167 bp). The intensity of the RIZ1 band is greatest in the pcDNA3.1(+)/RIZ1 group. M: Marker (D2000); N: Blank control; P: pcDNA3.1(+); R: pcDNA3.1(+)/RIZ1; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

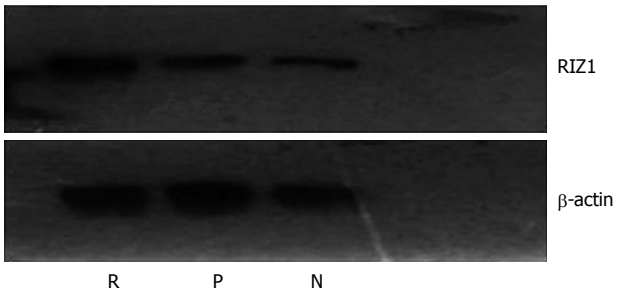

Figure 8 Western blot image for RIZ1 protein expression with β-actin as the control. The density of the RIZ1 band in the pcDNA3.1(+)/RIZ1 group is higher than in the other groups, indicating increased expression. R: pcDNA3.1(+)/RIZ1; N: Blank control; P: pcDNA3.1(+).

pression genes, therefore failure of RIZ1 expression can lead to the development of many forms of cancer[19-25]. Our group has carried out a number of studies on the *RIZ1* gene[11,12]; however, further research is required. One key research area is the introduction of a foreign gene into the tumor tissue, which is then able to express stably; eukaryotic expression vector is an ideal choice. The liposome mediated method was adopted because it has the advantages of high transfection efficiency, low immunogenicity, simple manipulation, and can be applied to a wide variety of cells.

We constructed the *RIZ1* gene eukaryotic expression vector pcDNA3.1(+)/RIZ1 using an established molecular biology technique; empty plasmid, pcDNA3.1(+), was used as a negative control. After transfection into ESCC cell line TE13, *in vivo* experiments and gene chip analyses were carried out, as described in Materials and Methods. Our results showed that the xenograft in nude mice had a slower growth rate, and lower tumor volume and mass, in the pcDNA3.1(+)/RIZ1 group than in the blank control and pcDNA3.1(+) groups. This suggests that RIZ1 has a restraining effect on ESCC tumor growth. In contrast, the growth curves for the blank control and pcDNA3.1(+) groups were approximately parallel, indicating that these groups had no restraining effect. Secondly, after transfection, the gene expression profile of the cell line TE13 underwent extensive changes, with a total of 2123 differentially expressed genes, including 1163 downregulated and 960 upregulated. We found that many of these genes are involved in cell development, lymphocyte costimulatory, immune system development, and interestingly, in the supervision of viral replication. This is consistent with the results from Dąbrowski *et al*[13] who reported that low-risk type human papilloma virus may be one of the auxiliary activated or carcinogenic factors in ESCC occurrence and development. Vaiphei *et al*[26] also found that the infection rates of human papilloma virus in ESCC patients was as high as 87%, especially in patients with two or more types of phenotypic mixed infection. Persson *et al*[27] reported that HIV infection increases the risk of esophageal cancer. All these reports indicate that the occurrence and development of esophageal cancer may be related to virus infection; however, the pathophysiological mechanisms are poorly understood and require further research.

In conclusion, we propose that the occurrence of esophageal cancer is a consequence of widespread alterations in gene expression, involving multiple functions and signaling pathways with roles in tumor development, some of which are synergistic or antagonistic. We also speculate that the most probable signaling pathways in ESCC affected by these genes are the cytokine receptor interaction and transforming growth factor pathways.

Therefore, we recommend that future research should be directed towards better understanding of the relationship between the *RIZ1* gene and ESCC and the mechanism and role of virus infection in ESCC occurrence and development.

COMMENTS

Background

Esophageal cancer is one of the most common forms of cancer in the world and is a leading cause of cancer deaths in many developing countries. China is a country with a high incidence of esophageal cancer, and the pathological type is mainly the squamous cell carcinoma, which is different from the Western countries where adenocarcinoma is reported to be the main pathological type. To date, the mechanisms of esophageal cancer are unclear. Tumor occurrence and development are regulated by a variety of oncogenes and tumor suppressor genes, including the putative tumor suppressor gene, retinoblastoma protein-interacting zinc finger gene 1 (*RIZ1*).

Research frontiers

RIZ1 expression is lower in esophagus carcinoma and is related to methylation of CpG islands. In addition, by constructing human RIZ1 eukaryotic expression vectors to transfect human esophageal squamous cell carcinoma (ESCC) cell line TE13, the authors were able to report that upregulation of RIZ1 can recover tumor suppression activity and that treatment of cell line TE13 by methyltransferase inhibitor 5-aza-CdR reverses the methylation status of the promoter region.

Innovations and breakthroughs

In order to investigate RIZ1-mediated changes in gene expression of esophageal cancer, the authors compared the gene expression profile of TE13 cells transfected with RIZ1 with that of negative control cells. The resulting changes in oncogenicity were analyzed *in vitro* and by animal experimentation. They found that the development and progression of esophageal cancer are related with the inactivation of RIZ1. In addition, virus infection may also be an important factor.

Applications

RIZ1 is one of the most effective tumor suppression genes, therefore failure of RIZ1 expression can lead to the development of ESCC, which is expected to be a molecular biological parameter for early diagnosis.

Peer review

This paper reports that the development and progression of esophageal cancer is relevant to the inactivation of RIZ1. In addition, virus infection may also be an important factor.

REFERENCES

1 **Dong SW**, Ma L, Xu N, Yan HQ, Liu HY, Li YW, Zhang P. Research on the reactivation of Syk expression caused by the inhibition of DNA promoter methylation in the lung cancer. *Neoplasma* 2011; **58**: 89-95 [PMID: 21067271 DOI: 10.4149/neo_2011_01_89]

2 **Ma L**, Dong S, Zhang P, Xu N, Yan H, Liu H, Li Y, Zhou Q. The relationship between methylation of the Syk gene in the promoter region and the genesis of lung cancer. *Clin Lab* 2010; **56**: 407-416 [PMID: 21086786]

3 **Dong SW**, Zhang P, Zhong RR, Liu Q. Expression of putative tumor suppressor gene spleen tyrosine kinase in esophageal squamous cell carcinoma. *Clin Lab* 2013; **59**: 647-653 [PMID: 23865365]

4 **Buyse IM**, Shao G, Huang S. The retinoblastoma protein binds to RIZ, a zinc-finger protein that shares an epitope with the adenovirus E1A protein. *Proc Natl Acad Sci USA* 1995; **92**: 4467-4471 [PMID: 7538672]

5 **Braig M**, Schmitt CA. Oncogene-induced senescence: putting the brakes on tumor development. *Cancer Res* 2006; **66**: 2881-2884 [PMID: 16540631 DOI: 10.1158/0008-5472.CAN-05-4006]

6 **Sasaki O**, Meguro K, Tohmiya Y, Funato T, Shibahara S, Sasaki T. Altered expression of retinoblastoma protein-interacting zinc finger gene, RIZ, in human leukaemia. *Br J Haematol* 2002; **119**: 940-948 [PMID: 12472571 DOI: 10.1046/j.1365-2141.2002.03972.x]

7 **Akahira J**, Suzuki F, Suzuki T, Miura I, Kamogawa N, Miki Y, Ito K, Yaegashi N, Sasano H. Decreased expression of RIZ1 and its clinicopathological significance in epithelial ovarian carcinoma: correlation with epigenetic inactivation by aberrant DNA methylation. *Pathol Int* 2007; **57**: 725-733 [PMID: 17922684 DOI: 10.1111/j.1440-1827.2007.02169.x]

8 **He L**, Yu JX, Liu L, Buyse IM, Wang MS, Yang QC, Nakagawara A, Brodeur GM, Shi YE, Huang S. RIZ1, but not the alternative RIZ2 product of the same gene, is underexpressed in breast cancer, and forced RIZ1 expression causes G2-M cell cycle arrest and/or apoptosis. *Cancer Res* 1998; **58**: 4238-4244 [PMID: 9766644]

9 **Chadwick RB**, Jiang GL, Bennington GA, Yuan B, Johnson CK, Stevens MW, Niemann TH, Peltomaki P, Huang S, de la Chapelle A. Candidate tumor suppressor RIZ is frequently involved in colorectal carcinogenesis. *Proc Natl Acad Sci USA* 2000; **97**: 2662-2667 [PMID: 10688904 DOI: 10.1073/pnas.040579497]

10 **Tam W**, Gomez MF, Chadburn A, Knowles DM, Houldsworth J. Lack of A563G (I188V) missense mutation in RIZ/PRDM2 in human diffuse large B-cell lymphomas. *Genes Chromosomes Cancer* 2007; **46**: 416-418 [PMID: 17205536 DOI: 10.1002/gcc.20409]

11 **Dong SW**, Cui YT, Zhong RR, Liang DC, Liu YM, Wang YG, He Z, Wang S, Liang SJ, Zhang P. Decreased expression of retinoblastoma protein-interacting zinc-finger gene 1 in human esophageal squamous cell cancer by DNA methylation. *Clin Lab* 2012; **58**: 41-51 [PMID: 22372344]

12 **Dong SW**, Zhang P, Liu YM, Cui YT, Wang S, Liang SJ, He Z, Sun P, Wang YG. Study on RIZ1 gene promoter methylation status in human esophageal squamous cell carcinoma. *World J Gastroenterol* 2012; **18**: 576-582 [PMID: 22363126 DOI: 10.3748/wjg.v18.i6.576]

13 **Dąbrowski A**, Kwaśniewski W, Skoczylas T, Bednarek W, Kuźma D, Goździcka-Józefiak A. Incidence of human papilloma virus in esophageal squamous cell carcinoma in patients from the Lublin region. *World J Gastroenterol* 2012; **18**: 5739-5744 [PMID: 23155315 DOI: 10.3748/wjg.v18.i40.5739]

14 **Enzinger PC**, Mayer RJ. Esophageal cancer. *N Engl J Med* 2003; **349**: 2241-2252 [PMID: 14657432 DOI: 10.1056/NEJMra035010]

15 **Chen BS**, Wang MR, Xu X, Cai Y, Xu ZX, Han YL, Wu M. Transglutaminase-3, an esophageal cancer-related gene. *Int J Cancer* 2000; **88**: 862-865 [PMID: 11093806]

16 **Metzger R**, Schneider PM, Warnecke-Eberz U, Brabender J, Hölscher AH. Molecular biology of esophageal cancer. *Onkologie* 2004; **27**: 200-206 [PMID: 15138356 DOI: 10.1159/000076913]

17 **Montesano R**, Hollstein M, Hainaut P. Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review. *Int J Cancer* 1996; **69**: 225-235 [PMID: 8682592]

18 **Qin YR**, Wang LD, Fan ZM, Kwong D, Guan XY. Comparative genomic hybridization analysis of genetic aberrations associated with development of esophageal squamous cell carcinoma in Henan, China. *World J Gastroenterol* 2008; **14**: 1828-1835 [PMID: 18350619 DOI: 10.3748/wjg.14.1828]

19 **Liu L**, Shao G, Steele-Perkins G, Huang S. The retinoblastoma interacting zinc finger gene RIZ produces a PR domain-lacking product through an internal promoter. *J Biol Chem* 1997; **272**: 2984-2991 [PMID: 9006946 DOI: 10.1074/jbc.272.5.2984]

20 **Steele-Perkins G**, Fang W, Yang XH, Van Gele M, Carling T, Gu J, Buyse IM, Fletcher JA, Liu J, Bronson R, Chadwick RB, de la Chapelle A, Zhang X, Speleman F, Huang S. Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein-methyltransferase superfamily. *Genes Dev* 2001; **15**: 2250-2262 [PMID: 11544182 DOI: 10.1101/

gad.870101]
21 **Hasegawa Y**, Matsubara A, Teishima J, Seki M, Mita K, Usui T, Oue N, Yasui W. DNA methylation of the RIZ1 gene is associated with nuclear accumulation of p53 in prostate cancer. *Cancer Sci* 2007; **98**: 32-36 [PMID: 17052263 DOI: 10.1111/j.1349-7006.2006.00338.x]
22 **Lal G**, Padmanabha L, Smith BJ, Nicholson RM, Howe JR, O'Dorisio MS, Domann FE. RIZ1 is epigenetically inactivated by promoter hypermethylation in thyroid carcinoma. *Cancer* 2006; **107**: 2752-2759 [PMID: 17103461 DOI: 10.1002/cncr.22325]
23 **Gazzerro P**, Abbondanza C, D'Arcangelo A, Rossi M, Medici N, Moncharmont B, Puca GA. Modulation of RIZ gene expression is associated to estradiol control of MCF-7 breast cancer cell proliferation. *Exp Cell Res* 2006; **312**: 340-349 [PMID: 16356493 DOI: 10.1016/j.yexcr.2005.11.002]
24 **Tokumaru Y**, Nomoto S, Jerónimo C, Henrique R, Harden S, Trink B, Sidransky D. Biallelic inactivation of the RIZ1 gene in human gastric cancer. *Oncogene* 2003; **22**: 6954-6958 [PMID: 14534544 DOI: 10.1038/sj.onc.1206403]
25 **Fang W**, Piao Z, Buyse IM, Simon D, Sheu JC, Perucho M, Huang S. Preferential loss of a polymorphic RIZ allele in human hepatocellular carcinoma. *Br J Cancer* 2001; **84**: 743-747 [PMID: 11259086 DOI: 10.1054/bjoc.2000.1667]
26 **Vaiphei K**, Kochhar R, Bhardawaj S, Dutta U, Singh K. High prevalence of human papillomavirus in esophageal squamous cell carcinoma: a study in paired samples. *Dis Esophagus* 2013; **26**: 282-287 [PMID: 22676445 DOI: 10.1111/j.1442-2050.2012.01365.x]
27 **Persson EC**, Shiels MS, Dawsey SM, Bhatia K, Anderson LA, Engels EA. Increased risk of stomach and esophageal malignancies in people with AIDS. *Gastroenterology* 2012; **143**: 943-950.e2 [PMID: 22796240 DOI: 10.1053/j.gastro.2012.07.013]

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ORIGINAL ARTICLE

Cytokeratin 8 is increased in hepatitis C virus cells and its ectopic expression induces apoptosis of SMMC7721 cells

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Abstract

AIM: To investigate cytokeratin 8 (*CK8*) overexpression during hepatitis C virus (HCV) infection and its pathogenesis, and the effect of ectopic *CK8* expression on hepatoma cell lines.

METHODS: We successfully established an *in vitro* HCV cell culture system (HCVcc) to investigate the different expression profiles of *CK8* in Huh-7-HCV and Huh-7.5-HCV cells. The expression of *CK8* at the mRNA level was determined by real-time polymerase chain reaction (RT-PCR). The expression of *CK8* at the protein level was evaluated by Western blotting. We then constructed a eukaryotic expression combination vector containing the coding sequence of human full length *CK8* gene. *CK8* cDNA was amplified by reverse transcription-PCR and inserted into pEGFP-C1 and the positive clone pEGFP-*CK8* was obtained. After confirming the sequence, the recombinant plasmid was transfected into SMMC7721 cells with lipofectamine2000 and *CK8* expression was detected using inverted fluorescence microscopy, RT-PCR and Western blotting. Besides, we identified biological function of *CK8* on SMMC7721 cells, including cell proliferation, cell cycle and apoptosis detection.

RESULTS: RT-PCR showed that the expression level of *CK8* in Huh-7-HCV and Huh-7.5-HCV cells was 2.88 and 2.95 times higher than in control cells. Western blot showed that *CK8* expression in Huh-7-HCV and Huh-7.5-HCV cells was 2.53 and 3.26 times higher than that in control cells, respectively. We found that *CK8* at mRNA and protein levels were both significantly increased in HCVcc. *CK8* was up-regulated in SMMC7721 cells. *CK8* expression at the mRNA level was significantly upregulated in SMMC7721/pEGFP-*CK8* cells. *CK8* expression in SMMC7721/ pEGFP-*CK8* cells was 2.69 times higher than in SMMC7721 cells, and was 2.64 times higher than in SMMC7721/pEGFP-C1 cells. *CK8* expression at the protein level in SMMC7721/pEGFP-*CK8* cells was 2.46 times higher than in SMMC7721 cells, and was 2.29 times higher than in SMMC7721/pEGFP-C1 cells. Further analysis demonstrated that forced expression of *CK8* slowed cell growth and induced apoptosis of SMMC7721 cells.

CONCLUSION: *CK8* up-regulation might have a functional role in HCV infection and pathogenesis, and could be a promising target for the treatment of HCV infection.

Key words: Hepatitis C virus cell culture system; Cytoker-

atin 8; Up-regulation; Eukaryotic expression; Apoptosis

Core tip: In this study, we observed that cytokeratin 8 (*CK8*) levels are elevated in hepatitis C virus (HCV) cell culture system and its ectopic expression decreased the proliferation and induced apoptosis of SMMC7721 cells. *CK8* up-regulation might have a functional role in HCV infection and pathogenesis, and could be a promising target for the treatment of HCV infection.

Sun MZ, Dang SS, Wang WJ, Jia XL, Zhai S, Zhang X, Li M, Li YP, Xun M. Cytokeratin 8 is increased in hepatitis C virus cells and its ectopic expression induces apoptosis of SMMC7721 cells. *World J Gastroenterol* 2013; 19(37): 6178-6187 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6178.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6178

INTRODUCTION

Hepatitis C virus (HCV) infection is a significant global healthcare burden[1]. Current estimation suggests that a minimum of 3% of the world's population is chronically infected, with a prevalence of up to 170 million people[2,3]. However, the mechanism of HCV infection is not fully understood. Recently, the development of HCV replicon technology has accelerated the understanding of the mechanism underlying HCV infection[4,5]. It has been reported that there were more than 100 abnormal expression proteins in HCV infected cells and hepatitis C patients[6-10]. Studies determining the changes in protein expression associated with HCV infection will help elucidate host/virus interactions, and provide further insight to HCV pathogenesis.

Cytokeratin 8 (*CK8*) is the major component of the intermediate filament cytoskeleton, belonging to the type-Ⅱ keratin, and is primarily expressed in the epithelia of liver, intestine, and exocrine pancreas[11,12]. *CK8* plays a crucial role in maintaining the structural integrity and the mechanical properties of cells[13]. Recent studies have suggested that *CK8* is involved in several liver diseases. *CK8* knock-out mice develop liver hemorrhage and are more susceptible to liver injury[14,15]. Some variants of *CK8* are associated with disease severity and progression in patients with chronic liver diseases[16,17]. Thus, we hypothesized that *CK8* contributed to cellular pathological processes and the infection and pathogenesis of HCV, leading to liver injury and chronic liver diseases.

In this study, we established an *in vitro* HCV cell culture system (HCVcc) and investigated whether HCV affects *CK8* levels. Simultaneously, we established eukaryotic expression recombination vector containing the full length coding sequence of *CK8*, then transfected into hepatoma cells *in vitro* and investigated the biological and functional role of *CK8* in hepatoma cells.

MATERIALS AND METHODS

Construction and identification of HCVcc

Huh-7 and Huh-7.5 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 0.1 mmol/L nonessential amino acids and 1 × penicillin-streptomycin-glutamine. Plasmid pFL-J6/JFH, containing a chimeric full length HCV genome, was kindly provided by Professor Charles M Rice from Rockefeller University. Plasmid pFL-J6/JFH, containing a single Xba I restriction site and T7 RNA polymerase start site, is the chimera of HCV J6 strain (5'-NCR-NS2) and JFH strain (NS3-3'-NCR). Subsequently, plasmid pFL-J6/JFH encoding the full length HCV chimeric genome was transcribed to HCV RNA *in vitro*. HCVcc was established by electroporation of HCV RNA into Huh-7 and Huh-7.5 cells.

Huh7 and Huh-7.5 were used as negative controls of HCVcc. Huh-7-HCV and Huh-7.5-HCV cells were maintained under the same condition as Huh-7 and Huh-7.5 cells. Cells were cultured in an incubator at 37 ℃ supplemented with 5% CO_2. During the cell culture, the supernatant of cell culture was collected at 24, 72 and 96 h after electroporation in order to determine the HCV copies. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine HCV copy number. At approximately 72 h after transfection, cells were washed three times with 1 × phosphate-buffered saline (PBS) and then harvested. In addition, indirect immunofluorescence was used to observe the expression of HCV core protein. Mouse monoclonal HCV core protein antibody (Novus Biologicals, United States) was used as the primary antibody, and goat anti-mouse conjugated with Fluorescein Isothiocyanate (FITC) was used as the secondary antibody. The harvested cells were fixed with 3% glutaraldehyde at 4 ℃ for 24 h, then washed twice by 0.1 mol/L arsenic acid dimethyl sodium buffer (pH 7.4) at 4 ℃, fixed by 1% osmium tetroxide for 1 h, gradient acetone dehydration, embedded by Epon812, sliced by ultra-thin LKB-V slicer. H-7650 transmission electron microscope (HITACHI, Japan) was also used to observe the morphology of the viral particles and intracellular ultrastructure changes.

Total RNA isolation, cDNA synthesis and RT-PCR

Total RNA was extracted from cells by TRIzol reagent (Invitrogen, United States) according to the manufacturer's protocol. A two-step reverse transcription PCR was performed. The first-strand cDNA was synthesized from 1 μg of total RNA with AMV Reverse Transcriptase[b] (TAKARA, Japan). To investigate the expression of *CK8* at the mRNA level, the expression of *CK8* and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) genes was quantified by RT-PCR, and GAPDH was used as an internal control. A total of 20 ng cDNA was used as template in the reaction. All RT-PCR assays were performed

Table 1 Primers used for real-time polymerase chain reaction and high fidelity

Name	Forward primer (5′-3′)	Reverse primer (5′-3′)
CK8 (172 bp)	AGCTGGAGTCTCGCCTGGAA	TGTGCCTTGACCTCAGCAATG
GAPDH (138 bp)	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA
CK8 (1465 bp)	ATGTCGACATGTCCATCAGGGTGAC	TAGGATCCCTTGGGCAGGACGTC

CK8: Cytokeratin 8; PCR: Polymerase chain reaction; GAPDH: Glyceraldehydes-3-phosphate dehydrogenase.

in triplicate using SYBR green incorporation method with Bio-Rad IQ5 Multicolor RT-PCR Detection System (Bio-Rad, United States) based on the manufacture's protocol. Table 1 shows the sequences of the primer sets for *CK8* and GAPDH. Briefly, following a denaturation at 95 ℃ for 5 s, RT-PCR was carried out with 50 cycles at a melting temperature of 95 ℃ for 30 s, an annealing temperature of 65 ℃ for 30 s, and an extension temperature of 72 ℃ for 10 s. Data analysis was performed using the Sequence Detector System software. The relative quantification was calculated by the $2^{-\Delta\Delta Ct}$ method with GAPDH as the housekeeping gene and the control cells as the baseline, and the results were expressed as fold-change.

Protein extraction, SDS-PAGE and Western blotting

Total proteins were prepared by RIPA cell lysate. Proteins of interest were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% polyacrylamide gel, and 1 mg/mL protein was loaded onto a SDS-PAGE gel. Proteins were transferred to nitrocellulose membranes and then detected by Western blotting under the recommended conditions. Mouse anti-human *CK8* IgG (Abcam, United States) was used as the primary antibody, goat anti-mouse IgG conjugated with horseradish peroxidase (HRP) was used as the secondary antibody, and GAPDH was used as the control. The antigen-antibody complex was detected by an enhanced chemiluminescence (ECL) kit following the manufacturer's protocol. The experiments were repeated in triplicate. The chemiluminescent signal of each band was analyzed by gel image analysis system (Syngene, United States).

Construction of pEGFP-CK8 recombination vector

The *Bam*H I and *Sal* I restriction sites were introduced into the *CK8* coding sequence (CDS) by high fidelity PCR (Thermo, United States). Sequences for the primers are listed in Table 1, with the amplified product being 1465 bp. The *CK8* CDS was purified by gel extraction. *CK8* CDS and pEGFP-C1 vector (TAKARA, Japan) were digested respectively by the restriction enzyme *Sal* I and *Bam*H I (TAKARA, Japan). The digestion products were examined on 1% agarose gel by electrophoresis. The ligation reaction (Ligation Kit, TAKARA, Japan) was carried out between both of the DNA fragments, followed by transformation into competent *Escherichia coli* DH5α cells at 37 ℃ overnight (12-16 h). Colony selection was performed by PCR, and the amplicons were examined on 1% agarose gel by electrophoresis. Plasmid extraction (E.Z.N.A.® Endo-Free Plasmid Midi Kit, Omega, United States) was carried out for positive colonies, and then sequenced and matched by Blast method.

Transfection of pEGFP-CK8 vector into SMMC 7721 cells

SMMC7721 cells were seeded in 6-well plates in 4 mL of growth medium for 24 h prior to transfection. In each well, 0.8×10^5-4.0×10^5 adherent cells were seeded. Four microgram (4.0 μg) of DNA (pEGFP-*CK8* vector or pEGFP-C1 vector) was diluted in 250 μL of serum-free DMEM. Lipofectamine2000 (Millipore, United States) was added (10 μL) to the diluted DNA and mixed immediately by pipetting. The mixture was incubated for 25 min at room temperature. The lipofectamine2000/DNA mixture (500 μL) was added dropwise to the four wells containing the pEGFP-*CK8* plasmid, and another two wells to control cells containing the pEGFP-C1 plasmid. The plate was then gently rocked to achieve even distribution of the complexes and incubated at 37 ℃ in a 5% CO_2 incubator.

Detection assay

The expression and distribution of *CK8* was observed under an inverted fluorescence microscope (Nikon eclipse Ti, Japan) 24 h after transfection. Forty-eight hours after transfection, cellular RNA and total cellular proteins were determined by RT-PCR and Western blotting, respectively. Total RNA was extracted from SMMC7721, SMMC7721/ pEGFP-C1, and SMMC7721/pEGFP-*CK8* cells by TRIzol reagent. Total proteins were prepared by RIPA cell lysate. Real time PCR assays (SYBR® *Premix Ex Taq*™ Ⅱ, TAKARA, Japan) were performed in triplicate with Bio-Rad iQ5 Multicolor RT-PCR Detection System according to the manufacture's protocol. Rabbit anti-human IgG (Santa, United States) was used as the primary antibody, goat anti-rabbit IgG conjugated with HRP was used as the secondary antibody and β-actin (Abcam, United States) was used as control. Cells were collected after 24, 48 and 72 h transfection to perform a proliferation assay by MTT reaction (MTT cell proliferation Assay kit, Trevigen, United States). Cells were also collected 48 h after transfection to detect apoptosis (Annexin V-FITC Apoptosis Detection Kit, Abcam, United States) using Flow Cytometry (guava easyCyte HT, Millipore, United States).

Statistical analysis

All experiments were performed in triplicate. Representative graphical data are presented as mean ± SD. Statistical analyses were performed using the SPSS 10.0 software

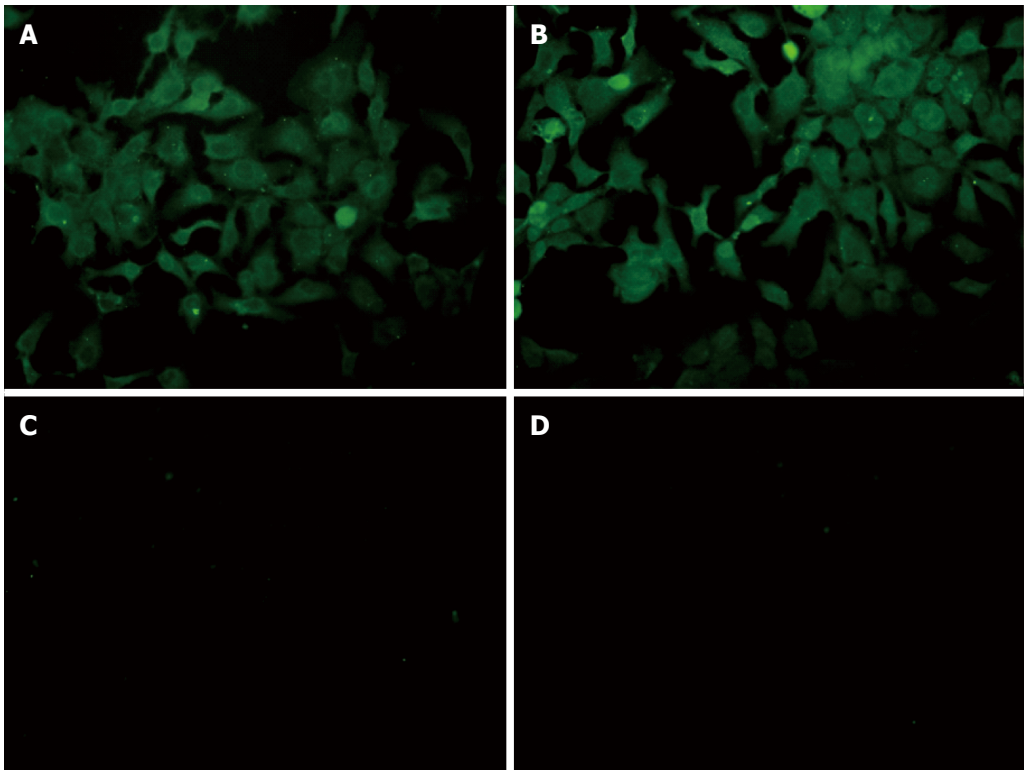

Figure 1 Indirect immunofluorescence detection of hepatitis C virus core proteins (400 ×). A: Huh-7-HCV cells appeared bright green fluorescent, they are HCV core proteins which were labeled with GFP; B: Huh-7.5-HCV cells appeared bright green fluorescent, they are HCV core proteins which were labeled with GFP; C and D: In Huh-7 and Huh-7.5 control cells, there were no green fluorescent, HCV core proteins were not expressed in them.

Table 2 Detection of hepatitis C virus at RNA level in transfected cellular supernatant

	HCV RNA in supernatant of Huh-7-HCV cells	HCV RNA in supernatant of Huh-7.5-HCV cells
24 h	5.73×10^5	9.48×10^5
48 h	1.38×10^6	6.40×10^6
72 h	3.00×10^4	9.29×10^4
96 h	6.62×10^3	1.43×10^4

HCV: Hepatitis C virus.

package (SPSS Inc.). We used Student's *t* test. *P* values below 0.05 were considered to be significant.

RESULTS

Detection of HCV RNA copies, HCV core protein, and HCV particles

We determined HCV RNA copy number by performing qRT-PCR of viral supernatants obtained from HCV-transfected cells. High-level viral copies in the supernatant of transfected cells were observed at different time-points and reached its peak value at 48 h after transfection (Table 2). Indirect immuno-fluorescence also showed high expression of HCV core protein in the HCV-transfected cells. Huh-7-HCV and Huh-7.5 HCV cells were also labeled with GFP, further indicating that HCV core protein has been expressed in these cells compared to control cells (Figure 1). Transmission electron microscopy (TEM) revealed a large number of enveloped or unenveloped virus-like particles (VLPs) in HCVcc. Some characteristic structures of *Flaviviridae* virus infection were observed, including an increased number of endoplasmic reticulum, mitochondrial swelling, cristae disappearance, and cytoplasmic vacuolar structures. Also, a large number of HCV nucleocapsid-like particles of inclusion body were presented in HCVcc cells (Figure 2). Viral-like particles were not seen in the control cells. Moreover, hyperplasia, vacuolar membrane structure, and formation of inclusion bodies were not observed in the control cells.

Increased CK8 levels in HCVcc cells by RT- PCR

Extracted total cellular RNA was examined by electrophoresis on a 0.8% non-denaturing agarose gel. A 172 bp fragment of *CK8* was successfully amplified by PCR without unspecific amplification. The melting and amplification curves of *CK8* expression indicated that the primers were properly designed. *CK8* expression in Huh-7-HCV cells was 2.88 times higher than that in Huh-7 cells, and *CK8* expression in Huh-7.5-HCV cells was 2.95 times higher than that in Huh-7.5 cells (Figure 3). Therefore, *CK8* was significantly highly expressed in HCVcc cells.

Increased CK8 levels determined by Western blotting of HCVcc cells

By Western blotting, we showed that the ratio of *CK8*/GAPDH was 0.079 ± 0.004 and 0.031 ± 0.003 in Huh-7-HCV cells and Huh-7 cells, respectively, which was 2.53

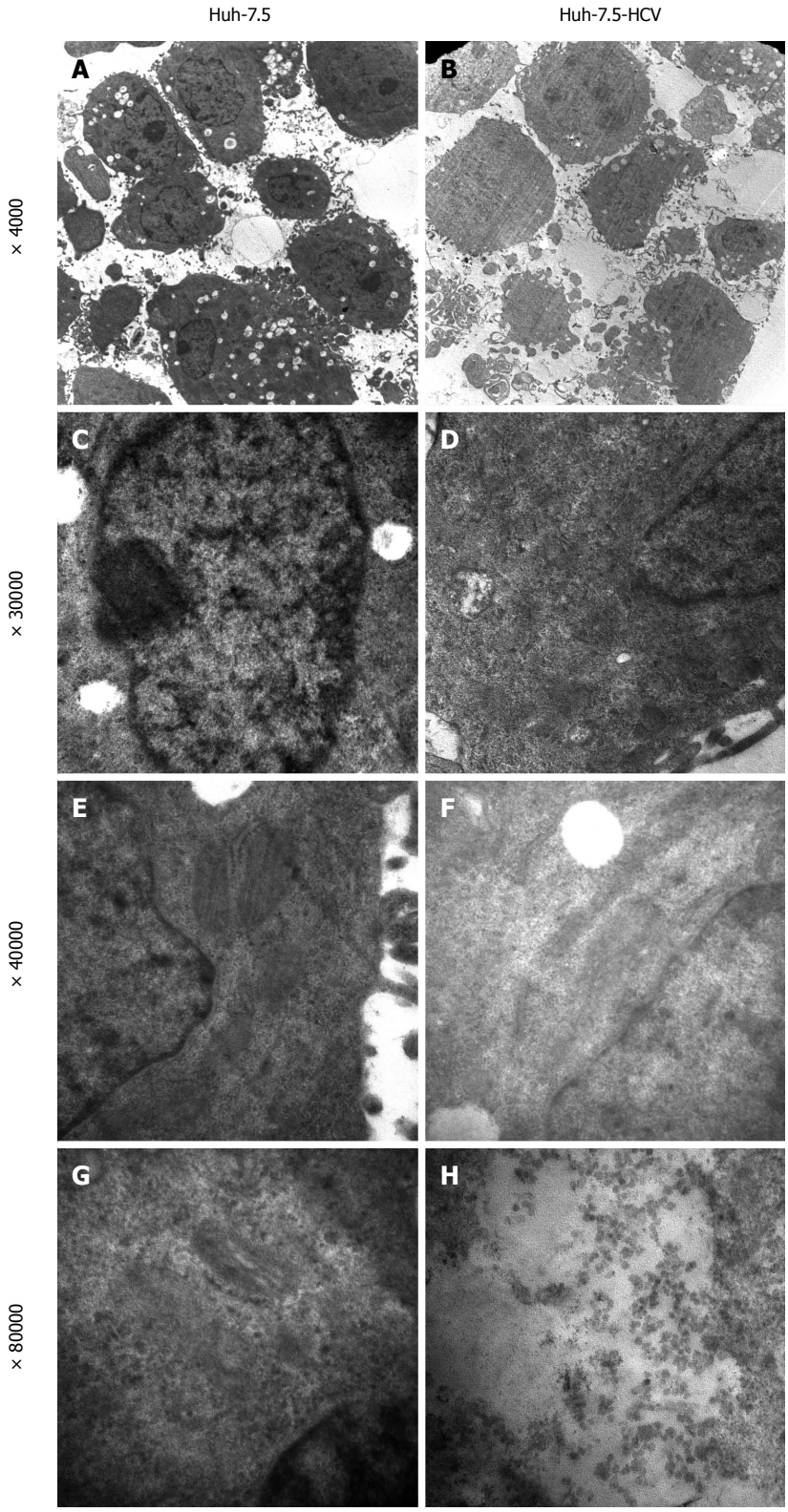

Figure 2 Transmission electron microscopy of hepatitis C virus-transfected Huh7.5 cells (× 4000, × 30000, × 40000, × 80000). A, C, E, G: Control human hepatoma cells, no virus-like particles, mitochondrial and endoplasmic reticulum are normal; B, D, F, H: HCV-transfected human hepatoma cells, human hepatoma cells have large deformed nuclei, and cultured cells prone to exist large vacuoles; D shows mitochondrial swelling and cristae disappearance; F shows the rough endoplasmic reticulum increased; H shows spherical structures of electron density, diameter is between 30-50 nm.

times higher. Furthermore, the ratio of *CK8*/GAPDH was 0.105 ± 0.004 in Huh-7.5-HCV cells, which was significantly higher than in Huh-7.5 cells (0.032 ± 0.002) and expression was 3.26 times higher (Figure 4). Therefore, we confirmed that HCVcc cells do have increased *CK8* expression.

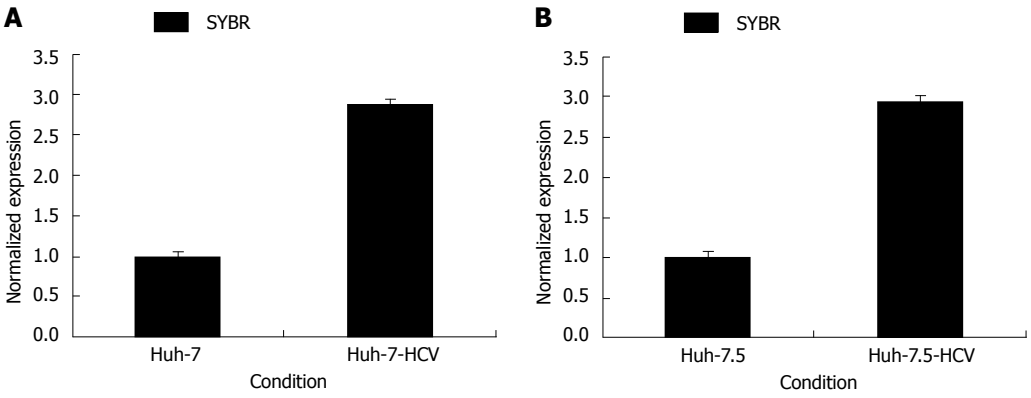

Figure 3 Relative cytokeratin 8 mRNA expression in Huh-7 and Huh-7- hepatitis C virus cells (A), or Huh-7.5 and Huh-7.5- hepatitis C virus cells (B).

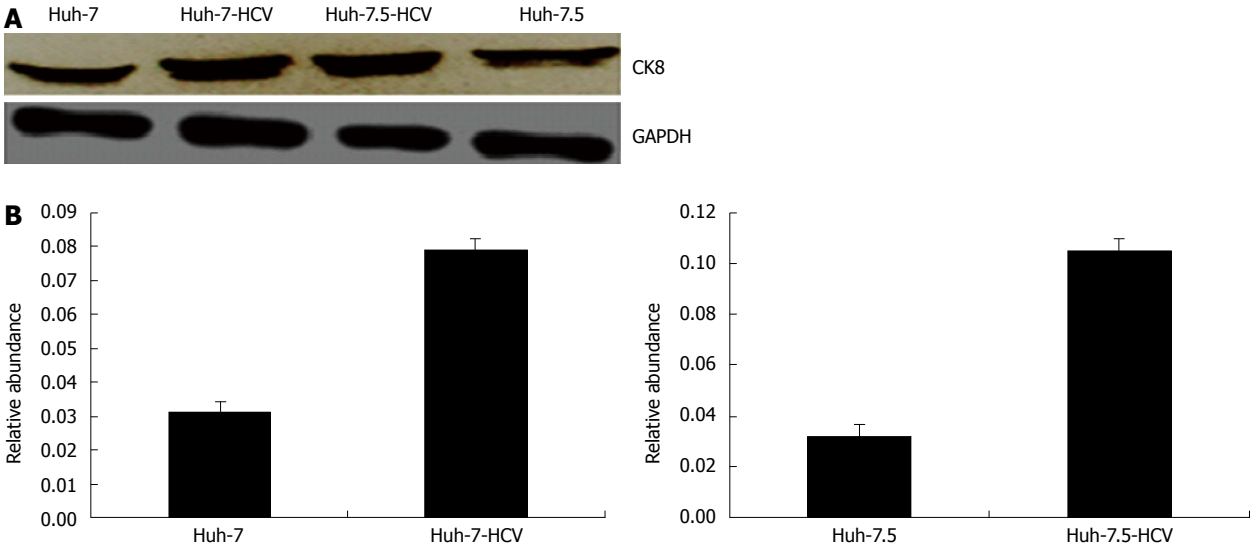

Figure 4 Cytokeratin 8 expression determined at the protein level by Western blotting in Huh-7 and Huh-7-hepatitis C virus cells (A), or Huh-7.5 and Huh-7.5-hepatitis C virus cells (B). HCV: Hepatitis C virus; CK8: Cytokeratin 8; GAPDH: Glyceraldehydes-3-phosphate dehydrogenase.

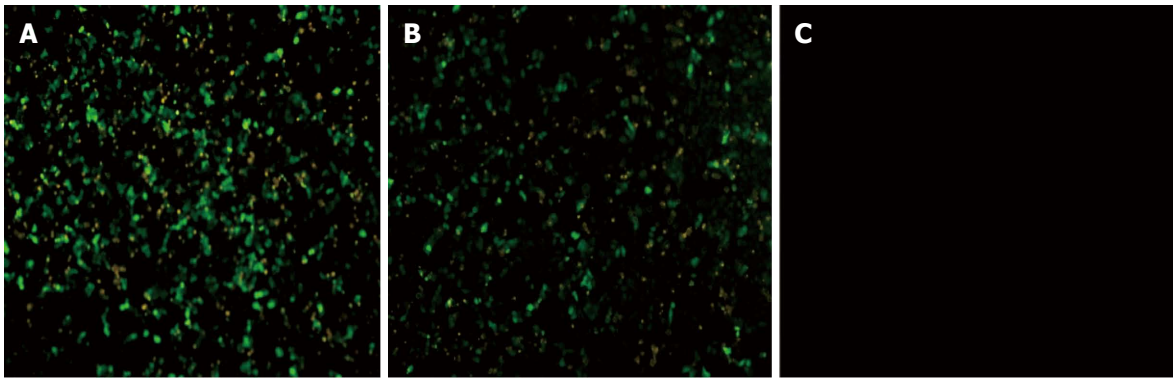

Figure 5 Inverted fluorescence microscopic observation 24 h after cytokeratin 8 transfection (× 200). A: SMMC7721 cells transfected by pEGFP-CK8 recombination vector; B: SMMC7721 cells transfected by pEGFP-C1 vector; C: SMMC7721 cells without transfection. CK8: Cytokeratin 8.

Inverted fluorescence microscopic observation

We next ectopically expressed *CK8* in SMMC7721 cells. We confirmed the overexpression of *CK8* in cells under inverted fluorescence microscope 24 h after transfection. Since the *CK8* expression vector contains an EGFP marker, we observed that SMMC7721/pEGFP-C1 and SMMC7721/pEGFP-*CK8* cells appeared bright green compared to control SMMC7721 cells (Figure 5). This data indicated that ectopic expression of *CK8* was achieved in SMMC7721 cells.

CK8 mRNA expression by qRT- PCR

The $2^{-\Delta\Delta Ct}$ value of *CK8* mRNA levels in SMMC7721, SMMC7721/pEGFP-C1, and SMMC7721/pEGFP-*CK8*

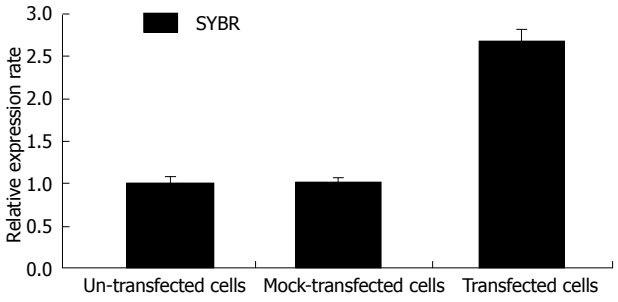

Figure 6 Cytokeratin 8 relative expression at the mRNA level.

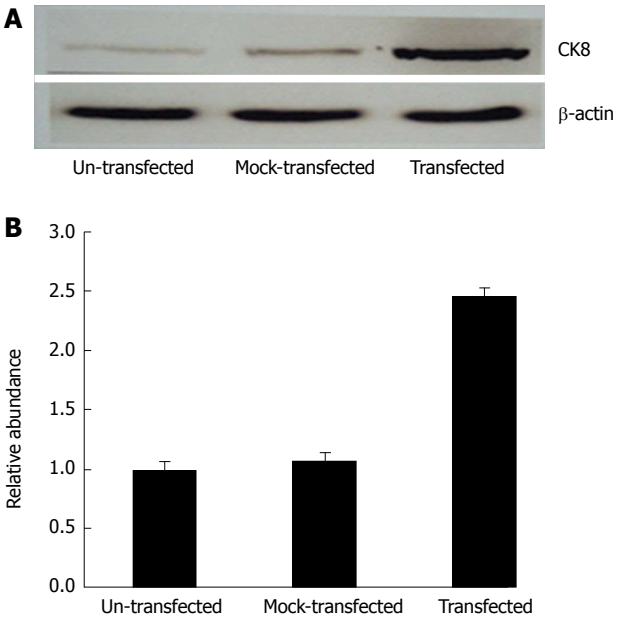

Figure 7 Cytokeratin 8 expression at the protein level 48h after transfection. CK8: Cytokeratin 8.

cells are shown in Figure 6. Beta-actin was used as the housekeeping gene, while SMMC7721 cells were used for baseline detection. The results were expressed as fold-change. *CK8* expression at the mRNA level was significantly upregulated in SMMC7721/pEGFP-*CK8* cells. *CK8* expression in SMMC7721/pEGFP-*CK8* cells was 2.69 times higher than in SMMC7721 cells, and was 2.64 times higher than in SMMC7721/pEGFP-C1 cells.

Ectopic expression of CK8 determined by Western blot analysis

Using Western blotting, we compared the chemiluminescent signals of *CK8* and β-actin in SMMC7721, SMMC7721/pEGFP-C1, and SMMC7721/pEGFP-*CK8* cells. The ratio between *CK8* and β-actin were reflective changes in *CK8* expression. *CK8* expression in SMMC7721/pEGFP-*CK8* cells was 2.46 times higher than in SMMC7721 cells, and 2.29 times higher than in SMMC7721/pEGFP-C1 cells. This demonstrated that ectopic expression of *CK8* was observed at the protein level in SMMC7721/pEGFP-*CK8* cells (Figure 7). Therefore, we confirmed that *CK8* expression was increased

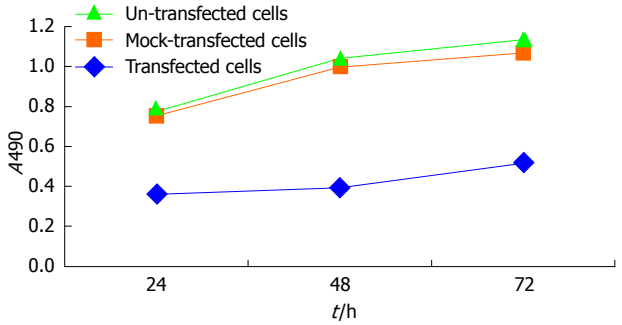

Figure 8 Growth chart after transfection in three groups of cells.

in SMMC7721 cells after transfection with pEGFP-*CK8* vector.

Effects of ectopic CK8 overexpression on cell proliferation

Using MTT detection, we determined the effects of ectopic *CK8* expression on SMMC7721 cells 72 h after transfection. *CK8* overexpression decreased the growth and proliferation of SMMC7721 cells compared to control cells and mock-transfected cells (Figure 8). This data indicated that ectopic *CK8* expression decreased cell growth and proliferation of SMMC7721 cells.

Effects of ectopic CK8 expression on the apoptosis of SMMC7721 cells

We determined the effects of ectopic *CK8* expression on the apoptosis of SMMC7721 cells 48 h after transfection. Using flow cytometry, ectopic *CK8* expression increased the apoptotic rate of SMMC7721 cells, compared to untransfected and mock-transfected cells (Figure 9).

DISCUSSION

In this study, we established a full-length HCV genomic replication in Huh-7 and Huh-7.5 cells. Lohmann *et al*[18] reported that subgenomic HCV RNA replicons are capable of autonomously replicating in Huh7 cells. These dicistronic replicons include the neomycin-resistant gene, making them selectable by G418, and most or all of the viral nonstructural genes[19,20]. This system provides a novel and powerful tool for the study of HCV replication mechanisms and for study of the interaction between host and viral factors involved in viral progression[21,22]. In our study, we transfected Huh-7 and Huh-7.5 cells to express HCV RNA and generated the HCVcc cell line. We used qRT-PCR, immunofluorescence, and TEM to detect HCV RNA, HCV core protein, and HCV particles, respectively. The results confirmed that HCV expression in Huh-7 and Huh-7.5 cells led to the production of HCV particles.

CK8 is a cytoskeletal intermediate filament protein that abundantly expresses in hepatocytes to maintain cell integrity, and prevent mechanical and non-mechanical cell injury[23,24]. Previous studies showed that *CK8* was upregulated in HBV-infected liver tissues fromp21-HBx mice[25],

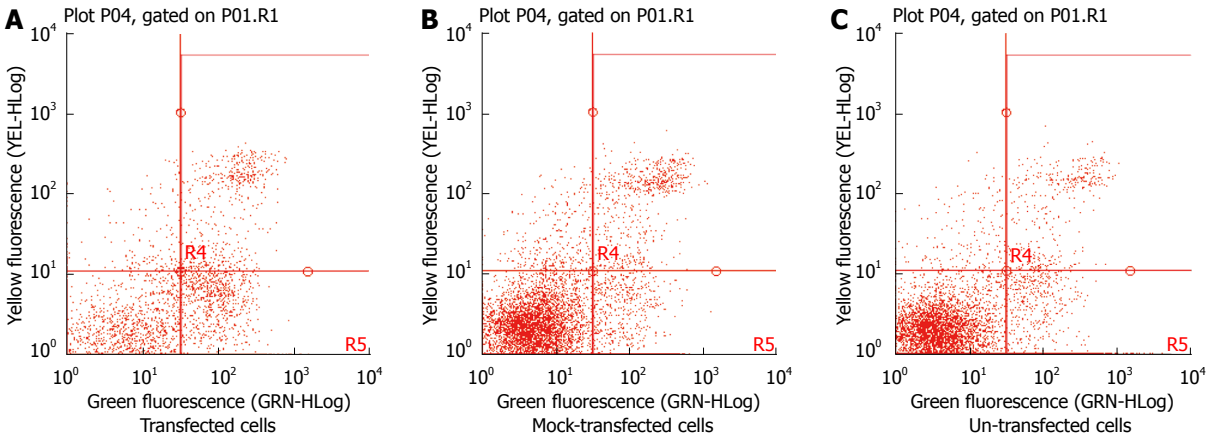

Figure 9 Cell flow cytometry. A: SMMC7721/ pEGFP-cytokeratin 8 cells; B: SMMC7721/ pEGFP-C1 cells; C: SMMC7721 cells without transfection. Cells were collected and washed twice with PBS, suspended in 200 μL binding buffer and 10 μL annexin V-FITC for 20 min in the dark, and thereafter, 300 μL binding buffer and 5 μL propidium iodide (PI) were added to each sample. The apoptotic cells were determined using a flow cytometer by staining with Annexin V-FITC. Representative dot plots show annexin V-FITC staining in cells. Results are representative of 3 independent experiments. FITC: Fluorescein isothiocyanate; PBS: Phosphate-buffered saline.

and that its upregulation contributed to the development and progression of HCC-induced HBV. Tai DI found that *CK8* was focally positive in a patient with a malignant liver patient infected with HCV[26]. Toivola *et al*[17] found that in chronic HCV infection, *CK8* phosphorylation is a progression marker during HCV progression and regression. Furthermore, Strnad *et al*[27] found that a number of *CK8* gene variants are increased in patients with chronic HCV infection. However, it is unclear about the relation between *CK8* expression and HCVcc cells. We observed a concomitant increase in *CK8* levels, which was confirmed by RT-PCR and Western blot analysis. *CK8* mRNA expression in Huh-7-HCV and Huh-7.5-HCV cells was 2.88 and 2.95 times higher than in Huh-7 and Huh-7.5 cells, respectively. At the protein level, *CK8* expression was 2.53 and 3.26 times higher in Huh-7-HCV and Huh-7.5-HCV cells, respectively, than Huh-7 and Huh-7.5 cells. This suggests that HCV up-regulates *CK8* expression in HCVcc cells, and that *CK8* expression is significantly associated with HCV.

CK8 plays a role in maintaining cellular structural integrity, signal transduction, and cellular differentiation[28-32]. Snider NT demonstrated that acetylation of *CK8* was up-regulated in diabetic human livers[33]. We showed that HCV up-regulates *CK8* expression in HCVcc cells. However, the biological function of ectopic *CK8* in tumor cells is not fully elucidated. To further investigate the biological function of aberrant *CK8* expression, we cloned the full length CDS of *CK8* to establish the eukaryotic expression recombination vector pEGFP-*CK8*. To study the biological function of increased *CK8* on cells independently, we chose another cell line called SMMC7721 cells in our laboratory. SMMC7721 cells were transfected by pEGFP-*CK8* recombination vector, and under an inverted fluorescence microscope we observed the expression and distribution of GFP-tagged *CK8*. In addition, by RT-PCR and Western blot analysis, we found that *CK8* mRNA levels in SMMC7721/pEGFP-*CK8* cells was 2.69 and 2.64 times higher than in SMMC7721 cells and SMMC7721/pEGFP-C1 cells, respectively. At the protein level, *CK8* expression in SMMC7721/pEGFP-*CK8* cells was 2.46 and 2.29 times higher than in SMMC7721 and SMMC7721/pEGFP-C1 cells, respectively. These observations showed that *CK8* gene was transcribed and expressed in SMMC7721 cells.

CK8 abnormal expression and mutations can lead to acute or sub-acute liver injury and promote tumor cells apoptosis[34,35]. The persistent expression of *CK8* can induce tumor cell apoptosis through a number of transcription factors that regulate a large number of oncogenes[36]. In SMMC7721 transfected by pEGFP-*CK8*, we further observed the biological effects of increased *CK8* on cells. We detected proliferation and apoptosis by MTT reaction and flow cytometry, respectively. We found that ectopic *CK8* expression decreased cell growth and proliferation, and increased apoptosis of SMMC7721 cells. Therefore, we concluded that the abnormal expression of *CK8* regulates cellular pathological injury. However, it is unclear what the mechanisms are by which *CK8* affects cell cycle and apoptosis. In conclusion, these results suggest *CK8* up-regulation might have a functional role during HCV infection and pathogenesis, and it could be a promising target for the treatment of HCV infection.

In summary, we successfully established and identified HCVcc and observed that *CK8* is upregulated in HCVcc. Overexpression of *CK8* in SMMC7721 cells inhibited cell proliferation and induced apoptosis. *CK8* could be a potential target for the treatment of HCV infection. Future studies will (1) identify the interactions of *CK8* with other proteins to mediate its effects; (2) assess how *CK8* expression regulates a number of known oncogenes in HCV; and (3) determine how *CK8* promotes apoptosis.

COMMENTS

Background

Currently, several proteins have been identified to be overexpressed during hepatitis C virus (HCV) infection and pathogenesis. Studies have suggested

that cytokeratin 8 (*CK8*) is closely related to a number of liver diseases. *CK8* knock-out mice develop liver hemorrhage and are more susceptible to liver injury. However, it remains unknown whether HCV affects *CK8* levels in their established *in vitro* HCV cell culture system (HCVcc) and the biological and functional role of *CK8* in hepatoma cells.

Research frontiers

It has been reported that there are more than 100 abnormal proteins expressed in HCV-infected cells and hepatitis C patients. Studies determining the changes in protein expression associated with HCV infection will help to elucidate host/virus interactions, and provide further insight to HCV pathogenesis. *CK8* plays a crucial role in maintaining the structural integrity and the mechanical properties of cells. Recent studies have suggested that *CK8* is involved in several liver diseases. Much interest is shown to understand *CK8* overexpression during HCV infection and to investigate the role of ectopic *CK8* expression in hepatoma cell lines.

Innovations and breakthroughs

In this study, the authors transfected Huh-7 and Huh-7.5 cells to express HCV RNA and generated the HCVcc cell line. Previous studies showed that *CK8* is upregulated in HBV-infected liver tissues from p21-HBx mice and in a patient with a malignant liver infected with HCV. However, it is unclear what the relation between *CK8* expression and HCVcc cells is. The authors observed a concomitant increase in *CK8* levels by real-time Polymerase chain reaction and Western blot analysis. The results show that HCV up-regulates *CK8* expression in HCVcc cells. However, the biological function of ectopic *CK8* in tumor cells is not fully elucidated. The authors found that ectopic *CK8* expression decreased cell growth and proliferation, and increased apoptosis of SMMC7721 cells. Therefore, the authors concluded that the abnormal expression of *CK8* regulates cellular pathological injury.

Applications

The results of this study suggest that *CK8* up-regulation might have a functional role during HCV infection and pathogenesis, and it could be a promising target for the treatment of HCV infection.

Peer review

This is a very well written manuscript. In this paper, the authors show the overexpression of *CK8* in an *in vitro* HCV cell culture system. Large-scale proteome analyses of the *in vitro* HCV infection model have also been performed. Thus new hopes characterize the HCV field and new advances are reasonably expected. Here, *CK8* is found up-regulated in Huh7 and Huh7.5 cells infected with chimeric full length HCV genome. The methodology is acceptable. The conclusions are interesting.

REFERENCES

1 **Park J**, Kang W, Ryu SW, Kim WI, Chang DY, Lee DH, Park do Y, Choi YH, Choi K, Shin EC, Choi C. Hepatitis C virus infection enhances TNFα-induced cell death via suppression of NF-κB. *Hepatology* 2012; **56**: 831-840 [PMID: 22430873 DOI: 10.1002/hep.25726]

2 **Mehrab-Mohseni M**, Sendi H, Steuerwald N, Ghosh S, Schrum LW, Bonkovsky HL. Legalon-SIL downregulates HCV core and NS5A in human hepatocytes expressing full-length HCV. *World J Gastroenterol* 2011; **17**: 1694-1700 [PMID: 21483629 DOI: 10.378/wjg.v17.i13.1694]

3 **Wang YZ**, Wang WB, Cao MM, Wang W, Zhao LJ, Xu G, Ren H, Qi ZT. Function of nonstructural 5A protein of genotype 2a in replication and infection of HCV with gene substitution. *World J Gastroenterol* 2011; **17**: 3398-3406 [PMID: 21876632 DOI: 10.3748/wjg.v17.i29.3398]

4 **Banaudha K**, Orenstein JM, Korolnek T, St Laurent GC, Wakita T, Kumar A. Primary hepatocyte culture supports hepatitis C virus replication: a model for infection-associated hepatocarcinogenesis. *Hepatology* 2010; **51**: 1922-1932 [PMID: 20512986 DOI: 10.1002/hep.23616]

5 **Boonstra A**, van der Laan LJ, Vanwolleghem T, Janssen HL. Experimental models for hepatitis C viral infection. *Hepatology* 2009; **50**: 1646-1655 [PMID: 19670425 DOI: 10.1002/hep.23138]

6 **Schaller T**, Appel N, Koutsoudakis G, Kallis S, Lohmann V, Pietschmann T, Bartenschlager R. Analysis of hepatitis C virus superinfection exclusion by using novel fluorochrome gene-tagged viral genomes. *J Virol* 2007; **81**: 4591-4603 [PMID: 17301154]

7 **Sogawa K**, Noda K, Umemura H, Seimiya M, Kuga T, Tomonaga T, Nishimura M, Kanai F, Imazeki F, Takizawa H, Yoneda M, Nakajima A, Tsutsumi M, Yokosuka O, Nomura F. Serum fibrinogen alpha C-chain 5.9 kDa fragment as a biomarker for early detection of hepatic fibrosis related to hepatitis C virus. *Proteomics Clin Appl* 2013; **7**: 424-431 [PMID: 23382097 DOI: 10.1002/prca.201200094]

8 **Tripathi LP**, Kambara H, Moriishi K, Morita E, Abe T, Mori Y, Chen YA, Matsuura Y, Mizuguchi K. Proteomic analysis of hepatitis C virus (HCV) core protein transfection and host regulator PA28γ knockout in HCV pathogenesis: a network-based study. *J Proteome Res* 2012; **11**: 3664-3679 [PMID: 22646850 DOI: 10.1021/pr300121a]

9 **Imakiire K**, Uto H, Sato Y, Sasaki F, Mawatari S, Ido A, Shimoda K, Hayashi K, Stuver SO, Ito Y, Okanoue T, Tsubouchi H. Difference in serum complement component C4a levels between hepatitis C virus carriers with persistently normal alanine aminotransferase levels or chronic hepatitis C. *Mol Med Rep* 2012; **6**: 259-264 [PMID: 22614103 DOI: 10.3892/mmr.2012.924]

10 **Qin S**, Zhou Y, Lok AS, Tsodikov A, Yan X, Gray L, Yuan M, Moritz RL, Galas D, Omenn GS, Hood L. SRM targeted proteomics in search for biomarkers of HCV-induced progression of fibrosis to cirrhosis in HALT-C patients. *Proteomics* 2012; **12**: 1244-1252 [PMID: 22577025 DOI: 10.1002/pmic.201100601]

11 **Fuchs E**, Weber K. Intermediate filaments: structure, dynamics, function, and disease. *Annu Rev Biochem* 1994; **63**: 345-382 [PMID: 7979242]

12 **Coulombe PA**, Omary MB. 'Hard' and 'soft' principles defining the structure, function and regulation of keratin intermediate filaments. *Curr Opin Cell Biol* 2002; **14**: 110-122 [PMID: 11792552]

13 **Busch T**, Armacki M, Eiseler T, Joodi G, Temme C, Jansen J, von Wichert G, Omary MB, Spatz J, Seufferlein T. Keratin 8 phosphorylation regulates keratin reorganization and migration of epithelial tumor cells. *J Cell Sci* 2012; **125**: 2148-2159 [PMID: 22344252 DOI: 10.1242/jcs.080127]

14 **Baribault H**, Price J, Miyai K, Oshima RG. Mid-gestational lethality in mice lacking keratin 8. *Genes Dev* 1993; **7**: 1191-1202 [PMID: 7686525]

15 **Loranger A**, Duclos S, Grenier A, Price J, Wilson-Heiner M, Baribault H, Marceau N. Simple epithelium keratins are required for maintenance of hepatocyte integrity. *Am J Pathol* 1997; **151**: 1673-1683 [PMID: 9403718]

16 **Zhong B**, Strnad P, Selmi C, Invernizzi P, Tao GZ, Caleffi A, Chen M, Bianchi I, Podda M, Pietrangelo A, Gershwin ME, Omary MB. Keratin variants are overrepresented in primary biliary cirrhosis and associate with disease severity. *Hepatology* 2009; **50**: 546-554 [PMID: 19585610 DOI: 10.1002/hep.23041]

17 **Toivola DM**, Ku NO, Resurreccion EZ, Nelson DR, Wright TL, Omary MB. Keratin 8 and 18 hyperphosphorylation is a marker of progression of human liver disease. *Hepatology* 2004; **40**: 459-466 [PMID: 15368451]

18 **Lohmann V**, Körner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 1999; **285**: 110-113 [PMID: 10390360]

19 **Shahid I**, Gull S, Ijaz B, Ahmad W, Ansar M, Asad S, Kausar H, Sarwar MT, Khan MK, Hassan S. Stable Huh-7 cell lines expressing non-structural proteins of genotype 1a of hepatitis C virus. *J Virol Methods* 2013; **189**: 65-69 [PMID: 23352716 DOI: 10.1016/j.jviromet.2013.01.005]

20 **Butt S**, Idrees M, Rehman IU, Ali L, Hussain A, Ali M, Ahmed N, Saleem S, Fayyaz M. Establishment of stable Huh-7 cell lines expressing various hepatitis C virus genotype 3a protein: an in-vitro testing system for novel anti-

HCV drugs. *Genet Vaccines Ther* 2011; **9**: 12 [PMID: 21711509 DOI: 10.1186/1479-0556-9-12]
21 **Kato T**, Date T, Miyamoto M, Furusaka A, Tokushige K, Mizokami M, Wakita T. Efficient replication of the genotype 2a hepatitis C virus subgenomic replicon. *Gastroenterology* 2003; **125**: 1808-1817 [PMID: 14724833]
22 **Dang SS**, Sun MZ, Yang E, Xun M, Ma L, Jia ZS, Wang WJ, Jia XL. Prohibitin is overexpressed in Huh-7-HCV and Huh-7.5-HCV cells harboring in vitro transcribed full-length hepatitis C virus RNA. *Virol J* 2011; **8**: 424 [PMID: 21896168 DOI: 10.1186/1743-422X-8-424]
23 **Sun Z**, Wang HQ, Liu ZH, Chang L, Chen YF, Zhang YZ, Zhang WL, Gao Y, Wan ZY, Che L, Liu X, Samartzis D, Luo ZJ. Down-regulated CK8 expression in human intervertebral disc degeneration. *Int J Med Sci* 2013; **10**: 948-956 [PMID: 23801880 DOI: 10.7150/ijms.5642]
24 **Andratschke M**, Luebbers CW, Johannson V, Schmitt B, Mack B, Zeidler R, Lang S, Wollenberg B, Gildehaus FJ. Biodistribution of 131I-labeled anti-CK8 monoclonal antibody in HNSCC in xenotransplanted SCID mice. *Anticancer Res* 2011; **31**: 3315-3321 [PMID: 21965741]
25 **Sun Q**, Wang Y, Zhang Y, Liu F, Cheng X, Hou N, Zhao X, Yang X. Expression profiling reveals dysregulation of cellular cytoskeletal genes in HBx-induced hepatocarcinogenesis. *Cancer Biol Ther* 2007; **6**: 668-674 [PMID: 17873514]
26 **Tai DI**, Shen YJ, Weng WH, Chen HW, Kang CC, Chen TC, Liao SK. New sarcomatoid cancer cell line SAR-HCV established from a hepatitis C virus-related liver tumour lesion. *Anticancer Res* 2011; **31**: 129-137 [PMID: 21273590]
27 **Strnad P**, Lienau TC, Tao GZ, Lazzeroni LC, Stickel F, Schuppan D, Omary MB. Keratin variants associate with progression of fibrosis during chronic hepatitis C infection. *Hepatology* 2006; **43**: 1354-1363 [PMID: 16729313]
28 **Fortier AM**, Asselin E, Cadrin M. Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through claudin1 up-regulation. *J Biol Chem* 2013; **288**: 11555-11571 [PMID: 23449973 DOI: 10.1074/jbc.M112.428920]
29 **Iyer SV**, Dange PP, Alam H, Sawant SS, Ingle AD, Borges AM, Shirsat NV, Dalal SN, Vaidya MM. Understanding the role of keratins 8 and 18 in neoplastic potential of breast cancer derived cell lines. *PLoS One* 2013; **8**: e53532 [PMID: 23341946 DOI: 10.137/journal.pone.0053532]
30 **Wang Y**, Zhu JF, Liu YY, Han GP. An analysis of cyclin D1, cytokeratin 5/6 and cytokeratin 8/18 expression in breast papillomas and papillary carcinomas. *Diagn Pathol* 2013; **8**: 8 [PMID: 23327593 DOI: 10.1186/1746-1596-8-8]
31 **Mathew J**, Loranger A, Gilbert S, Faure R, Marceau N. Keratin 8/18 regulation of glucose metabolism in normal versus cancerous hepatic cells through differential modulation of hexokinase status and insulin signaling. *Exp Cell Res* 2013; **319**: 474-486 [PMID: 23164509 DOI: 10.1016/j.yexcr.2012.11.011]
32 **Usachov V**, Nahon P, Lunova M, Ziol M, Rufat P, Sutton A, Beaugrand M, Strnad P. Keratin 8 variants are infrequent in patients with alcohol-related liver cirrhosis and do not associate with development of hepatocellular carcinoma. *BMC Gastroenterol* 2012; **12**: 147 [PMID: 23078008 DOI: 10.1186/1471-230X-12-147]
33 **Snider NT**, Leonard JM, Kwan R, Griggs NW, Rui L, Omary MB. Glucose and SIRT2 reciprocally mediate the regulation of keratin 8 by lysine acetylation. *J Cell Biol* 2013; **200**: 241-247 [PMID: 23358244 DOI: 10.1083/jcb.201209028]
34 **Ku NO**, Strnad P, Zhong BH, Tao GZ, Omary MB. Keratins let liver live: Mutations predispose to liver disease and crosslinking generates Mallory-Denk bodies. *Hepatology* 2007; **46**: 1639-1649 [PMID: 17969036]
35 **Wang Y**, He QY, Tsao SW, Cheung YH, Wong A, Chiu JF. Cytokeratin 8 silencing in human nasopharyngeal carcinoma cells leads to cisplatin sensitization. *Cancer Lett* 2008; **265**: 188-196 [PMID: 18353540 DOI: 10.1016/j.canlet.2008.02.015]
36 **Oshima RG**, Baribault H, Caulín C. Oncogenic regulation and function of keratins 8 and 18. *Cancer Metastasis Rev* 1996; **15**: 445-471 [PMID: 9034603]

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BRIEF ARTICLE

Screening pre-bariatric surgery patients for esophageal disease with esophageal capsule endoscopy

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Abstract

AIM: To determine if esophageal capsule endoscopy (ECE) is an adequate diagnostic alternative to esophagogastroduodenoscopy (EGD) in pre-bariatric surgery patients.

METHODS: We conducted a prospective pilot study to assess the diagnostic accuracy of ECE (PillCam ESO2, Given Imaging) *vs* conventional EGD in pre-bariatric surgery patients. Patients who were scheduled for bariatric surgery and referred for pre-operative EGD were prospectively enrolled. All patients underwent ECE followed by standard EGD. Two experienced gastroenterologists blinded to the patient's history and the findings of the EGD reviewed the ECE and documented their findings. The gold standard was the findings on EGD.

RESULTS: Ten patients with an average body mass index of 50 kg/m^2 were enrolled and completed the study. ECE identified 11 of 14 (79%) positive esophageal/gastroesophageal junction (GEJ) findings and 14 of 17 (82%) combined esophageal and gastric findings identified on EGD. Fisher's exact test was used to compare the findings and no significant difference was found between ECE and EGD (P = 0.64 for esophageal/GEJ and P = 0.66 for combined esophageal and gastric findings respectively). Of the positive esophageal/GEJ findings, ECE failed to identify the following: hiatal hernia in two patients, mild esophagitis in two patients, and mild Schatzki ring in two patients. ECE was able to identify the entire esophagus in 100%, gastric cardia in 0%, gastric body in 100%, gastric antrum in 70%, pylorus in 60%, and duodenum in 0%.

CONCLUSION: There were no significant differences in the likelihood of identifying a positive finding using ECE compared with EGD in preoperative evaluation of bariatric patients.

Key words: Capsule endoscopy; Bariatric; Moderate sedation; Esophagogastroduodenoscopy; Esophageal capsule endoscopy

Core tip: This is the first prospective study that shows in pre-bariatric patients, capsule endoscopy can be used to identify positive esophageal disorders when compared to a sedated esophagogastroduodenoscopy. Further studies are needed to help define the role of esophageal capsule endoscopy as a tool for pre-operative evaluation.

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INTRODUCTION

In patients with morbid obesity, surgery is a treatment option associated with good medium and long-term results with procedures such as gastric banding, sleeve gastrectomy, gastric bypass, and biliopancreatic diversion[1]. These operations can be performed laparoscopically in most obesity centers[2-4]. Preoperative esophagogastroduodenoscopy (EGD) is useful to detect pathological findings that might preclude or delay bariatric surgery[5].

Patients referred for bariatric surgery often have co-morbidities including obstructive sleep apnea, arterial hypertension, coronary heart disease or diabetes mellitus which puts them at risk for any procedure that involves conscious sedation. The risk of EGD in these patients includes aspiration, hypoxemia, hypoventilation, airway obstruction, vasovagal episodes, and arrhythmias. Esophageal capsule endoscopy (ECE) offers a less invasive diagnostic alternative in evaluating diseases of the esophagus. ECE does not require sedation and is therefore better tolerated by patients. Several studies have shown that ECE is an adequate alternative diagnostic method for esophageal variceal screening and diagnosis of Barrett's esophagus in patients with chronic gastroesophageal reflux[6-8]. Delvaux *et al*[9] concluded that capsule endoscopy showed a moderate sensitivity and specificity in detecting esophageal diseases such as esophagitis, hiatal hernias, varices and Barrett's esophagus. They determined the overall positive predictive value of capsule endoscopy was 80%. In this study we aim to determine if capsule endoscopy is adequate in identifying specific esophageal and gastric pathology for patients undergoing bariatric surgery as compared to EGD.

MATERIALS AND METHODS

This was a prospective pilot study. Patients from 2010 to 2012 who were scheduled to undergo bariatric surgery at University of California San Diego Medical Center and referred for pre-operative EGD were prospectively enrolled to assess the diagnostic accuracy of the ECE (PillCam ESO2, Given Imaging) *vs* conventional EGD. A total of ten patients were enrolled in the study. Patients were enrolled after Human Subjects Research Protection Committee approved consent was obtained. All patients underwent ECE followed by standard EGD performed under moderate sedation with fentanyl and versed All patients underwent ECE followed by standard EGD performed by a single endoscopist that was video recorded. Demographic data was collected on each patient including age, sex, weight in kilograms (kg), and body mass index (BMI) per patient's medical chart. Co-morbidities on each patient were documented including obstructive sleep apnea, coronary artery disease hypertension, type Ⅱ diabetes mellitus, chronic kidney disease, and non-alcoholic fatty liver disease (NAFLD). Two experienced gastroenterologists reviewed the ECE and EGD videos and documented their findings. Both gastroenterologists were blinded to patients' history. Findings on ECE were than compared with the findings on EGD. Findings were categorical variables where 0 represented a normal finding and 1 represented an abnormal finding. Abnormal findings included esophagitis, Schatzki ring, and hiatal hernia. Fisher's exact test was used to compare the findings between the two modalities with the findings on the EGD considered the gold standard.

RESULTS

Table 1 provides baseline demographic information and co-morbidities on each patient. The mean age was 46.2 years with the majority of patients being female (6/10). The average BMI was 50.12 kg/m^2 and average weight of 141.85 kg. Eight out of ten patients and nine out of ten patients suffered from suffered from obstructive sleep apnea and hypertension respectively. Forty percent of patients suffered from diabetes and 30% of patients had a diagnosis of NAFLD. Two out of ten patients had chronic kidney disease with a glomerular filtration rate of 55 and 48 mL/min/1.73 sqm.

Visualization of the esophagus, GE junction, and gastric body were seen 100% of the time on ECE. The antrum and pylorus were identified 70% and 60% of the time respectively. The gastric cardia and the duodenum were not able to be identified on any capsule endoscopies. The majority of the capsules (9/10) were retained in the stomach (Table 1).

Esophageal findings on EGD included Schatzki ring, hiatal hernia and esophagitis. Two patients were noted to have gastric findings. One patient was found to have mild gastropathy and the other patient was described as having a watermelon stomach at the pylorus.

ECE identified 11 of 14 (79%) positive esophageal/gastroesophageal junction (GEJ) findings and 14 of 17 (82%) combined esophageal and gastric findings identified on EGD. Correctly identified abnormal findings on ECE as seen on EGD in the esophagus included hiatal hernia in seven out of ten patients (Figure 1), Schatzki ring in one out of three patients and felinization rings were correctly identified in one patient on both ECE and EGD. Gastric findings seen on ECE as well as EGD included gastropathy in the body of the stomach in three out of three patients. Erosions were correctly identified on ECE and EGD in one patient. There was a trend toward agreement among ECE and EGD findings in the esophagus *vs* stomach (35/40 findings in agreement in the esophagus *vs* 30/40 findings in agreement in the

Table 1 Results of patient characteristics and visualization (*n* = 10) *n* (%)

Result	Value
Characteristics	
Mean age (yr) (median)	46.2 (46)
Female	6 (60)
Mean body mass index (kg/m^2) (median)	50.12 (48.1)
Weight (kg) (median)	141.85 (139.9)
Obstructive sleep apnea	8 (80)
Coronary artery disease	1 (10)
Hypertension	9 (90)
Diabetes mellitus	4 (40)
Chronic kidney disease	2 (20)
Non-alcoholic fatty liver disease	3 (30)
Visualization	
Mean esophageal transit time (min) (median)	3.997 (3.997)
Esophagus	10 (100)
Gastroesophageal junction	10 (100)
Gastric cardia	0 (0)
Gastric body	10 (100)
Gastric antrum	7 (70)
Pylorus	6 (60)
Duodenum	0 (0)

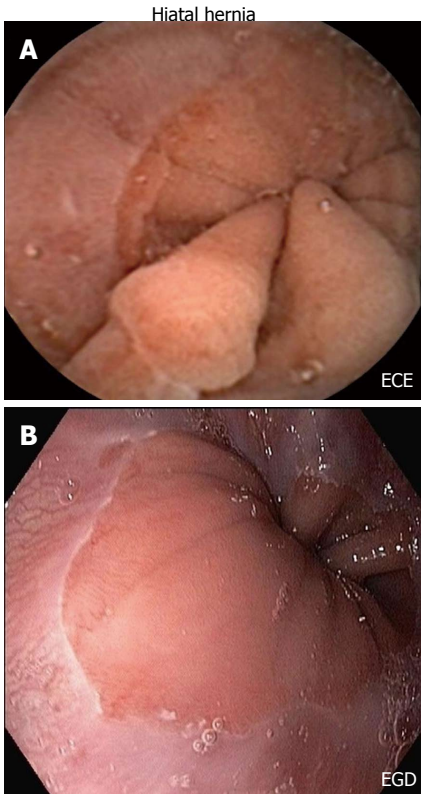

Figure 1 Comparison of esophageal capsule endoscopy *vs* esophagogastroduodenoscopy. Image of a hiatal hernia on esophageal capsule endoscopy (ECE, panel A) *vs* esophagogastroduodenoscopy (EGD, panel B). ECE was able to correctly abnormal findings in the esophagus such as hiatal hernias when compared to EGD.

stomach) (Figure 2A).

Fisher's exact test was used to compare the findings and no significant difference was found between ECE and EGD (P = 0.64 for esophageal/GEJ and P = 0.66 for combined esophageal and gastric findings respectively) (Figure 2B). Of the positive esophageal/GEJ findings, ECE failed to identify the following: hiatal hernia in two patients, mild esophagitis in two patients, and mild Schatzki ring in two patients. Conversely, ECE identified a Schatzki ring in one patient, an irregular Z-line in another patient, and a hiatal hernia in a third patient not identified on EGD. There were no adverse events related to ECE.

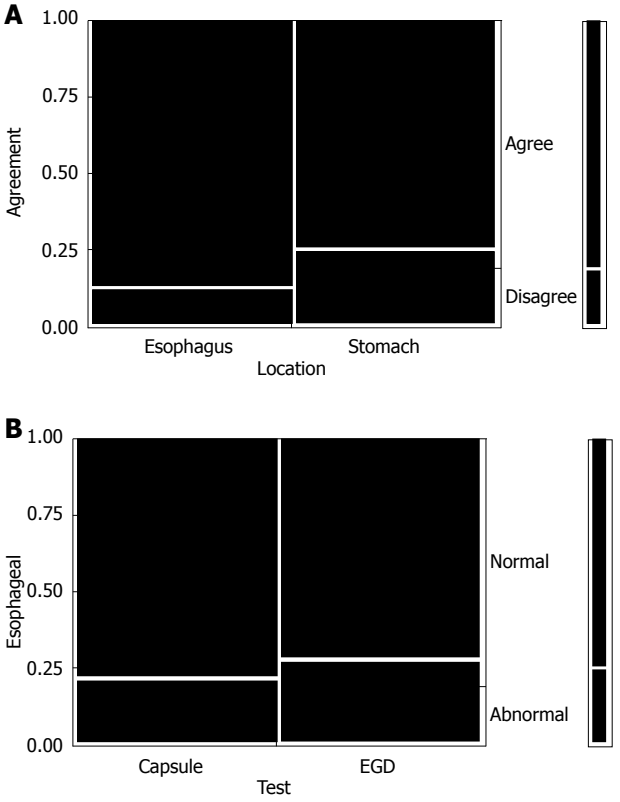

Figure 2 Inter-observer agreement between esophageal and gastric findings and results of esophageal findings on capsule *vs* esophagogastroduodenoscopy. A: There was a trend toward agreement among esophageal capsule endoscopy and esophagogastroduodenoscopy findings in the esophagus *vs* stomach (35/40 findings in agreement in the esophagus *vs* 30/40 findings in agreement in the stomach); B: There was no significant difference between esophageal capsule endoscopy and esophagogastroduodenoscopy (EGD) (P = 0.64) for combined esophageal and gastroesophageal junction findings on 2-tailed Fischer exact test (P = 0.66).

DISCUSSION

Bariatric surgery is increasingly performed to treat morbid obesity and its complications. Pre-operative EGD can help identify useful pathology that can negatively influence post-operative outcomes. However EGD is an invasive procedure requiring the use of conscious sedation that can carry risks of cardiopulmonary complications, hypoxemia, aspiration and cardiac arrhythmias[10-13]. Quine *et al*[10] showed in a prospective study of 14149 upper endoscopies that 31 patients experienced cardiopulmonary complications related to moderate sedation. Similar studies have shown the rate of cardiopulmonary complications to range from 1.3 per thousand to 5.4 per thousand[11,14]. Sharma *et al*[15] showed that the risk of developing unplanned cardiopulmonary events following

upper endoscopy was 0.6%. Multiple studies have determined that the risks of moderate sedation in patients undergoing endoscopy increase with co-morbidities such as advanced age, obstructive sleep apnea, underlying heart disease, and obesity[10-12,14,16]. Sharma *et al*[15] found that an increased American Society of Anesthesiologists classification (ASA class score) was attributed to an increase risk of developing cardiopulmonary complications. Odds ratio ranged from 1.1 with an ASA Ⅱ score to 1.8 with ASA class Ⅲ and 7.4 with class Ⅴ. Many bariatric surgery patients will generally have higher ASA scores.

Endoscopic capsule was initially approved by the Food and Drug Administration in 2001 for evaluation of the small bowel. It is increasingly being used for the evaluation of obscure GI bleeding, Crohns' disease, and suspected small bowel tumors[17-21]. Several studies have shown that capsule endoscopy can be used to identify esophageal disorders such as Barrett's esophagus/esophagitis, hiatal hernia, and esophageal varices[22-26].

Our study shows that in pre-bariatric patients, capsule endoscopy can be used to identify positive esophageal disorders when compared to a sedated EGD. Capsule endoscopy was correctly able to identify hiatal hernias, Schatzki rings and esophagitis when compared with EGD. However in our study ECE failed to identify gastric pathology the majority of the time. The gastric cardia was not visualized during ECE and the gastric antrum and pylorus were identified 30% and 40% of the time respectively. The duodenum also could not be correctly identified by ECE due to the short capsule recording time of 20 min.

Esophageal findings seen on ECE but not noted on EGD included a Schatzki ring in one patient, an irregular Z-line in another patient, and a hiatal hernia in a third patient. This might be explained by changes in body position between the upright ECE and left lateral decubitus EGD.

Further limitations of this pilot study include a small patient size. Only ten patients were enrolled in the trial. Despite this, results trended towards no significant difference in the likelihood of identifying a positive finding using ECE compared with EGD.

In conclusion, this is the first study to suggest that ECE may be a safer alternative than sedated EGD for evaluation of esophageal disorders prior to bariatric surgery. However ECE cannot consistently evaluate for gastric or duodenal pathology. Further studies are needed to help define the role of ECE as a tool for pre-operative evaluation.

COMMENTS

Background

In patients with morbid obesity, bariatric surgery is a treatment option associated with good medium and long-term results. Prior to surgery, many patients undergo an endoscopic examination to determine if they have any pathology to prevent or delay them from undergoing bariatric surgery. Esophagogastroduodenoscopy (EGD) however is an invasive procedure which comes with risks including the risk of sedation associated with morbidly obese patients. Small bowel capsule endoscopy has been used to identify pathology in the small bowel for years. Esophageal capsule endoscopy (ECE) uses the same technology to examine the esophagus and stomach. Several studies have shown that ECE is able to identify pathology and anatomic variations in the esophagus and stomach, and can be performed without the risks of moderate sedation. No studies to date have been done to determine whether capsule endoscopy is equivalent to EGD in screening pre-bariatric surgery patients.

Research frontiers

Small bowel capsule endoscopy was developed as a means to non-invasively examine the small bowel for pathology. ECE was subsequently developed and food and drug administration approved using the same technology to examine the esophagus and stomach. There have been several studies to suggest that ECE is comparable to EGD in the diagnosis of upper gastrointestinal disorders such as Barrett's esophagus and esophageal varices.

Innovations and breakthroughs

Small bowel capsule endoscopy is a useful tool to non-invasively examine the small bowel. ECE uses the same technology to examine the esophagus and stomach. Recent studies have shown that ECE is comparable EGD in diagnosing esophageal pathology such as esophagitis, Barrett's esophagus and esophageal varices as well as stomach pathology such as gastric cancers. However ECE has never been compared to EGD in pre-screening patients for pathology which may preclude them from bariatric surgery. This study is the first study to date to compare the two in this patient population.

Applications

The study results indicate that ECE may be a safer alternative than sedated EGD for evaluation of esophageal disorders prior to bariatric surgery, but cannot consistently evaluate for gastric or duodenal pathology. Further studies are needed to help define the role of ECE as a tool for pre-operative evaluation.

Terminology

Bariatric surgery: Surgery is performed on the stomach and/or intestines in order to facilitate weight loss in obese patients. This could be achieved either through restrictive alone or both restrictive and malabsorptive mechanisms. EGD: An imaging test that involves visually examining the lining of the esophagus, stomach, and upper duodenum with a flexible fiberoptic endoscope; ECE: A small camera inside a capsule shaped and sized like a pill which is used to take video images of the digestive tract to help in evaluation of symptoms such as gastrointestinal bleeding or abdominal pain; Moderate sedation: Drug induced consciousness (typically carried out with a combination of a narcotic such as fentanyl and benzodiazepine such as midazolam) during which patients respond purposefully to verbal commands, either alone or by light tactile stimulation. No interventions are needed to maintain a patent airway, patient is able to spontaneously breathe during moderate sedation. Used in a wide variety of medical procedures.

Peer review

The results suggest that ECE may be a safer alternative than sedated EGD for evaluation of esophageal disorders prior to bariatric surgery. The paper would be desirable to specify

REFERENCES

1 **Brolin RE**. Bariatric surgery and long-term control of morbid obesity. *JAMA* 2002; **288**: 2793-2796 [PMID: 12472304]

2 **Kueper MA**, Kramer KM, Kirschniak A, Königsrainer A, Pointner R, Granderath FA. Laparoscopic sleeve gastrectomy: standardized technique of a potential stand-alone bariatric procedure in morbidly obese patients. *World J Surg* 2008; **32**: 1462-1465 [PMID: 18368447 DOI: 10.1007/s00268-008-9548-2]

3 **Moy J**, Pomp A, Dakin G, Parikh M, Gagner M. Laparoscopic sleeve gastrectomy for morbid obesity. *Am J Surg* 2008; **196**: e56-e59 [PMID: 18954593 DOI: 10.1016/j.amjsurg.2008.04.008]

4 **Tice JA**, Karliner L, Walsh J, Petersen AJ, Feldman MD. Gastric banding or bypass? A systematic review comparing the two most popular bariatric procedures. *Am J Med* 2008; **121**: 885-893 [PMID: 18823860 DOI: 10.1016/j.amjmed.2008.05.036]

5 **Anderson MA**, Gan SI, Fanelli RD, Baron TH, Banerjee S, Cash BD, Dominitz JA, Harrison ME, Ikenberry SO, Jagan-

nath SB, Lichtenstein DR, Shen B, Lee KK, Van Guilder T, Stewart LE. Role of endoscopy in the bariatric surgery patient. *Gastrointest Endosc* 2008; **68**: 1-10 [PMID: 18577471 DOI: 10.1016/j.gie.2008.01.028]

6 **Eliakim R**, Sharma VK, Yassin K, Adler SN, Jacob H, Cave DR, Sachdev R, Mitty RD, Hartmann D, Schilling D, Riemann JF, Bar-Meir S, Bardan E, Fennerty B, Eisen G, Faigel D, Lewis BS, Fleischer DE. A prospective study of the diagnostic accuracy of PillCam ESO esophageal capsule endoscopy versus conventional upper endoscopy in patients with chronic gastroesophageal reflux diseases. *J Clin Gastroenterol* 2005; **39**: 572-578 [PMID: 16000923]

7 **Lin OS**, Schembre DB, Mergener K, Spaulding W, Lomah N, Ayub K, Brandabur JJ, Bredfeldt J, Drennan F, Gluck M, Jiranek GC, McCormick SE, Patterson D, Kozarek RA. Blinded comparison of esophageal capsule endoscopy versus conventional endoscopy for a diagnosis of Barrett's esophagus in patients with chronic gastroesophageal reflux. *Gastrointest Endosc* 2007; **65**: 577-583 [PMID: 17324414 DOI: 10.1016/j.gie.2006.06.035]

8 **Lu Y**, Gao R, Liao Z, Hu LH, Li ZS. Meta-analysis of capsule endoscopy in patients diagnosed or suspected with esophageal varices. *World J Gastroenterol* 2009; **15**: 1254-1258 [PMID: 19291827]

9 **Delvaux M**, Papanikolaou IS, Fassler I, Pohl H, Voderholzer W, Rösch T, Gay G. Esophageal capsule endoscopy in patients with suspected esophageal disease: double blinded comparison with esophagogastroduodenoscopy and assessment of interobserver variability. *Endoscopy* 2008; **40**: 16-22 [PMID: 18058656 DOI: 10.1055/s-2007-966935]

10 **Quine MA**, Bell GD, McCloy RF, Charlton JE, Devlin HB, Hopkins A. Prospective audit of upper gastrointestinal endoscopy in two regions of England: safety, staffing, and sedation methods. *Gut* 1995; **36**: 462-467 [PMID: 7698711]

11 **Arrowsmith JB**, Gerstman BB, Fleischer DE, Benjamin SB. Results from the American Society for Gastrointestinal Endoscopy/U.S. Food and Drug Administration collaborative study on complication rates and drug use during gastrointestinal endoscopy. *Gastrointest Endosc* 1991; **37**: 421-427 [PMID: 1833259]

12 **Lieberman DA**, Wuerker CK, Katon RM. Cardiopulmonary risk of esophagogastroduodenoscopy. Role of endoscope diameter and systemic sedation. *Gastroenterology* 1985; **88**: 468-472 [PMID: 3965335]

13 **Yen D**, Hu SC, Chen LS, Liu K, Kao WF, Tsai J, Chern CH, Lee CH. Arterial oxygen desaturation during emergent nonsedated upper gastrointestinal endoscopy in the emergency department. *Am J Emerg Med* 1997; **15**: 644-647 [PMID: 9375545]

14 **Silvis SE**, Nebel O, Rogers G, Sugawa C, Mandelstam P. Endoscopic complications. Results of the 1974 American Society for Gastrointestinal Endoscopy Survey. *JAMA* 1976; **235**: 928-930 [PMID: 128642]

15 **Sharma VK**, Nguyen CC, Crowell MD, Lieberman DA, de Garmo P, Fleischer DE. A national study of cardiopulmonary unplanned events after GI endoscopy. *Gastrointest Endosc* 2007; **66**: 27-34 [PMID: 17591470 DOI: 10.1016/j.gie.2006.12.040]

16 **Barkin JS**, Krieger B, Blinder M, Bosch-Blinder L, Goldberg RI, Phillips RS. Oxygen desaturation and changes in breathing pattern in patients undergoing colonoscopy and gastroscopy. *Gastrointest Endosc* 1989; **35**: 526-530 [PMID: 2599296]

17 **Adler DG**, Knipschield M, Gostout C. A prospective comparison of capsule endoscopy and push enteroscopy in patients with GI bleeding of obscure origin. *Gastrointest Endosc* 2004; **59**: 492-498 [PMID: 15044884]

18 **Fireman Z**, Mahajna E, Broide E, Shapiro M, Fich L, Sternberg A, Kopelman Y, Scapa E. Diagnosing small bowel Crohn's disease with wireless capsule endoscopy. *Gut* 2003; **52**: 390-392 [PMID: 12584221]

19 **Cobrin GM**, Pittman RH, Lewis BS. Increased diagnostic yield of small bowel tumors with capsule endoscopy. *Cancer* 2006; **107**: 22-27 [PMID: 16736516 DOI: 10.1002/cncr.21975]

20 **Pennazio M**, Santucci R, Rondonotti E, Abbiati C, Beccari G, Rossini FP, De Franchis R. Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. *Gastroenterology* 2004; **126**: 643-653 [PMID: 14988816]

21 **de Leusse A**, Vahedi K, Edery J, Tiah D, Fery-Lemonnier E, Cellier C, Bouhnik Y, Jian R. Capsule endoscopy or push enteroscopy for first-line exploration of obscure gastrointestinal bleeding? *Gastroenterology* 2007; **132**: 855-862; quiz 1164-1165 [PMID: 17324401 DOI: 10.1053/j.gastro.2006.12.002]

22 **Galmiche JP**, Sacher-Huvelin S, Coron E, Cholet F, Soussan EB, Sébille V, Filoche B, d'Abrigeon G, Antonietti M, Robaszkiewicz M, Le Rhun M, Ducrotté P. Screening for esophagitis and Barrett's esophagus with wireless esophageal capsule endoscopy: a multicenter prospective trial in patients with reflux symptoms. *Am J Gastroenterol* 2008; **103**: 538-545 [PMID: 18190647 DOI: 10.1111/j.1572-0241.2007.01731.x]

23 **Borobio E**, Fernández-Urién I, Elizalde I, Jiménez Pérez FJ. Hiatal hernia and lesions of gastroesophageal reflux disease diagnosed by capsule endoscopy. *Rev Esp Enferm Dig* 2009; **101**: 355-356 [PMID: 19527082]

24 **Ahn D**, Guturu P. Meta-analysis of capsule endoscopy in patients diagnosed or suspected with esophageal varices. *World J Gastroenterol* 2010; **16**: 785-786 [PMID: 20135731]

25 **Sánchez-Yagüe A**, Caunedo-Alvarez A, García-Montes JM, Romero-Vázquez J, Pellicer-Bautista FJ, Herrerías-Gutiérrez JM. Esophageal capsule endoscopy in patients refusing conventional endoscopy for the study of suspected esophageal pathology. *Eur J Gastroenterol Hepatol* 2006; **18**: 977-983 [PMID: 16894311 DOI: 10.1097/01.meg.0000230094.21911.f8]

26 **Bhardwaj A**, Hollenbeak CS, Pooran N, Mathew A. A meta-analysis of the diagnostic accuracy of esophageal capsule endoscopy for Barrett's esophagus in patients with gastroesophageal reflux disease. *Am J Gastroenterol* 2009; **104**: 1533-1539 [PMID: 19491867 DOI: 10.1038/ajg.2009.86]

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BRIEF ARTICLE

Acid and non-acid reflux in patients refractory to proton pump inhibitor therapy: Is gastroparesis a factor?

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Abstract

AIM: To determine whether an increased number and duration of non-acid reflux events as measured using the multichannel intraluminal impedance pH (MII-pH) is linked to gastroparesis (GP).

METHODS: A case control study was conducted in which 42 patients undergoing clinical evaluation for continued symptoms of gastroesophageal reflux disease (both typical and atypical symptoms) despite acid suppression therapy. MII-pH technology was used over 24 h to detect reflux episodes and record patients' symptoms. Parameters evaluated in patients with documented GP and controls without GP by scintigraphy included total, upright, and supine number of acid and non-acid reflux episodes (pH < 4 and pH > 4, respectively), the duration of acid and non-acid reflux in a 24-h period, and the number of reflux episodes lasting longer than 5 min.

RESULTS: No statistical difference was seen between the patients with GP and controls with respect to the total number or duration of acid reflux events, total number and duration of non-acid reflux events or the duration of longest reflux episodes. The number of non-acid reflux episodes with a pH > 7 was higher in subjects with GP than in controls. In addition, acid reflux episodes were more prolonged (lasting longer than 5 min) in the GP patients than in controls; however, these values did not reach statistical significance. Thirty-five patients had recorded symptoms during the 24 h study and of the 35 subjects, only 9% (n = 3) had a positive symptom association probability (SAP) for acid/non-acid reflux and 91% had a negative SAP.

CONCLUSION: The evaluation of patients with a documented history of GP did not show an association between GP and more frequent episodes of non-acid reflux based on MII-pH testing.

Key words: Gastroparesis; Non-acid gastroesophageal reflux; Acid gastroesophageal reflux; Multi-channel intraluminal impedance; Functional bowel disorder

Core tip: Gastroparesis (GP) has been thought to occur in about 8%-10% of patients who suffer from refractory gastroesophageal reflux disease (GERD). There have been no formal studies to date that have evaluated whether patients with refractory GERD additionally suffer from GP. Our study aimed to investigate whether patients who experience continued symptoms of GERD despite acid suppression therapy also concurrently have gastroparesis. By using multichannel intraluminal impedance pH technology, we were not able to find an association between patients with refractory GERD and gastroparesis.

Tavakkoli A, Sayed BA, Talley NJ, Moshiree B. Acid and non-acid reflux in patients refractory to proton pump inhibitor therapy: Is gastroparesis a factor? *World J Gastroenterol* 2013; 19(37): 6193-6198 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6193.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6193

INTRODUCTION

Gastroparesis (GP) is a chronic gastrointestinal (GI) motility disorder characterized by delayed gastric emptying in the absence of mechanical outlet obstruction[1]. The most common etiologies of GP include those secondary to chronic disease states such as diabetes mellitus and collagen-vascular diseases, post-surgical causes, medication induced, and idiopathic causes[1,2]. Symptoms of GP include early satiety, nausea, vomiting, abdominal pain, bloating, and gastroesophageal reflux[1-3]. Notably, GP has been rising in prevalence across the United States, which as a result, has led to an increase in GP related hospitalizations and increasing health care costs[1,4].

Gastroesophageal reflux disease (GERD) is a very common disease in the United States and has recently been show to be the most common GI diagnosis among clinical visits in the United States[5]. The mainstay of treatment for GERD includes proton pump inhibitors (PPIs) but several studies have shown that upwards of 40% of patients who suffer from heartburn have either a partial or complete lack of response to PPIs taken once a day[6-8]. A number of causes for PPI failure have been investigated, including medication non-compliance, undiagnosed functional bowel disorders, and GP[9].

GP has been reported to be present in a variable subset of patients who suffer from refractory GERD and studies have shown that anywhere between 8%-10% of patients with refractory GERD additionally suffer from GP[10-14]. It is unknown whether the hypothesized link between GP and refractory GERD actually exists in clinical practice. The aim of our study was to determine whether there is an increased number and duration of non-acid reflux events when comparing patients with GP and without GP using multichannel intraluminal impedance pH (MII-pH). Given the delayed gastric emptying of food in patients with GP, we hypothesized that patients with GP will experience a greater number of non-acid reflux episodes for a longer duration of time when compared to controls without GP.

MATERIALS AND METHODS

The University of Florida Health Science Center Institutional Review Board approved the collection and analysis of MII-pH data obtained in this study.

Patient population

From July 2009 to September 2010, all patients who underwent MII-pH analysis for continued symptoms of GERD despite PPI therapy were enrolled after signing informed consent (n = 66). The patients' health records were reviewed for evidence of a prior gastric emptying scintigraphy (GES) done at the University of Florida (n = 39) or the results were documented in a recent clinic note from a prior GES done at an outside institution (n = 3). Patients were then included in this study if a GES was previously done (abnormal at University of Florida if $t^{1/2}$ > 90 min) and they were scheduled to or had undergone a MII-pH study at the gastric motility laboratory at University of Florida. Patients were excluded from the study if they had no prior GES or history of one documented in a recent clinical note. However, patients were not excluded if they were currently on anti-acid therapy. All patients underwent a physical examination and upper endoscopy prior to undergoing the ambulatory MII-pH testing to exclude any mechanical obstruction.

Patients were considered refractory to PPI therapy at the discretion of the clinician who had referred them to receive MII-pH analysis. Patients were defined as "refractory" if they continued to have symptoms of GERD despite anti-acid therapy. All patients remained on their previously prescribed PPI therapy during the duration of the study.

MII-pH monitoring

Subjects presented to the motility laboratory after an overnight fast. The Sleuth ambulatory system (Sandhill Scientific, Inc; Highlands Ranch, CO, United States) was used to perform the impedance testing. The catheter has an antimony pH electrode measuring the pH 5 cm from the distal tip and 6 impedance electrodes that measures impedance at 3, 5, 7, 9, 15 and 17 cm above the distal tip. The catheter was placed transnasally with its tip 5 cm above the lower esophageal sphincter.

Subjects then underwent a 24-h monitoring in which their symptoms were electronically recorded and the patients kept a diary of their food intake. All 42 subjects were continued on their current acid suppression therapy for the entire duration of the study. All prokinetic agents, including bethanechol, domperidone, metoclopramide, and azithromycin were discontinued.

Symptoms recorded in the electrical diaries included both typical GERD symptoms (heartburn, regurgitation, and chest pain) and atypical GERD symptoms (cough, hoarseness, abdominal discomfort, nausea, belching, globus sensation, and dysphagia). After the 24-h ambulatory period, subjects returned to the motility laboratory where the data was transferred and analyzed using a single investigatory dedicated software (BioView Analysis; Sandhill Scientific, Inc.). Two investigators in the motility laboratory manually reviewed tracings and electronic diary entry of symptoms. Meal periods were marked and excluded from the analysis. Only liquid and mixed liquid/gas reflux episodes were evaluated for this study.

The parameters obtained from the MII-pH device included the total, upright, and supine number of acid and non-acid reflux episodes, the duration of acid and non-

Table 1 Demographics and esophagogastroduodenoscopy results among patients with gastroparesis and controls

Result	GP	Controls	*P* value
Demographics			
Age (mean ± SD, yr)	54 ± 14	51 ± 13	0.620
Female (*n*)	12	19	
Males (*n*)	4	7	
Gastric emptying scintigraphy			
$t^{1/2}$ time (mean ± SD, min)	134 ± 59	65 ± 13	0.0003
Comorbidities (*n*)			
GERD	16	26	
Irritable bowel syndrome	2	1	
Diabetes mellitus	1	2	
Hypothyroidism	1	4	
Chronic constipation	3	2	
Hepatitis C	1	1	
IBD	1	0	
Previous surgical procedures (*n*)			
Cholecystectomy	4	6	
Nissen Fundoplication	3	1	
EGD status (*n*)			
Normal EGD	6	11	
Gastritis (*H. pylori* negative)	7	6	
Atrophic gastritis	1	0	
Fundic gland polyp	2	0	
Hyperplastic polyp	0	1	
Antacid usage (*n*)			
H2 blocker therapy	2	5	
Daily	1	3	
Bid	1	1	
Tid	0	1	
PPI therapy (*n*)	14	15	
Daily	4	4	
Bid	9	10	
Tid	1	1	
Sucralfate (*n*)	2	3	
Antacids (*n*)	1	1	
No therapy (*n*)	0	3	

EGD: Esophagogastroduodenoscopy; GERD: Gastroesophageal reflux disease; IBD: Inflammatory bowel disease; *H. pylori*: *Helicobacter pylori*; PPI: Proton pump inhibitor.

acid reflux in a 24-h period, and the number of reflux episodes lasting longer than 5 min. The MII-pH detected reflux episodes that were classified as acidic when the esophageal pH fell below 4, non-acidic if the pH was between 4-7, and alkaline if the pH rose above 7.

Based on the MII-pH data, we evaluated each separate symptom and determined their association with a reflux episode. The symptom association probability (SAP) was electronically calculated using the BioView Analysis Software. The 24-h pH data was divided into 2-min segments and each of the 2 min segments were studied to determine whether a reflux event and a symptom occurred during that segment. A 2 × 2 table was made in which the number of two minute segments with and without reflux and with and without symptoms were tabulated. A χ^2 test was then used to determine whether the occurrence of the symptoms and reflux could have occurred by chance. The SAP was then calculated using the formula $SAP = (1 - P) \times 100\%$ and it was considered positive if > 95%[15].

Statistical analysis

The impedance pH data was compared using the Wilcoxon non-parametric analysis of variance for means and the 2-sided Fisher exact test for proportions. All values are expressed as a mean ± SD. The null hypothesis assumes that no significant difference in the number of non-acid reflux events will be seen when comparing impedance pH data between the GP group and the control group.

RESULTS

Demographics and medical conditions

The study included 42 participants chosen based on their GES results and then divided into two groups: subjects with gastroparesis and subjects with normal GES both undergoing MII-pH for continued symptoms of GERD despite PPI therapy.

The GP group included 16 patients with a mean age of 54 ± 14 years (age range: 24-76 years) with a mean GES half-time ($t^{1/2}$) of 134 ± 59 min (normal defined at UF as $t^{1/2}$ between 45-90 min). The control group consisted of 26 patients with a mean age of 51 ± 13 years (age range: 24-77 years) with a mean GES ($t^{1/2}$) of 65 ± 13 min (Table 1). Among the GP group, 6 patients had a normal esophagogastroduodenoscopy (EGD) and 7 patients had biopsy proven *Helicobacter pylori* (*H. pylori*) negative gastritis (Table 1). In the control group, 11 patients had a normal EGD and 6 patients had biopsy proven *H. pylori* negative gastritis (Table 1).

Symptoms on presentation among both the GP and control group included heartburn, bloating, nausea/vomiting chest pain, and hoarseness. Concurrent medical diagnoses included GERD, irritable bowel syndrome, type Ⅱ diabetes mellitus, and chronic constipation. Prior surgical procedures in both populations included cholecystectomy and Nissen fundoplication.

MII-pH data

No statistical difference was seen between subjects with GP and controls with respect to the total number and duration of acid reflux events [13.3 ± 17.1 (95%CI: 4.2-22.4) in GP *vs* 12.0 ± 14.8 (95%CI: 6.0-18.0) in controls, $P < 0.79$], total number and duration of non-acid reflux events [21.6 ± 24.6 (95%CI: 8.5-34.7) in GP *vs* 25.7 ± 29.3 (95%CI: 13.9-37.5) in controls, $P < 0.64$], or the total number and duration of reflux events [30.8 ± 36.5 (95%CI: 11.3-50.2) in GP *vs* 37.9 ± 35.7 (95%CI: 23.48-52.29) in controls, $P < 0.54$] (Figure 1). The number of non-acid reflux episodes with a pH > 7 were higher in subjects with GP [5.3 ± 5 (95%CI: 2.6-8.0) *vs* 4.5 ± 5.6 (95%CI: 2.3-6.9) in controls, $P < 0.67$] and the acid reflux episodes were more prolonged (lasting longer than 5 min) in the GP group [0.95 ± 2.0 (95%CI: -1.1-2.0) *vs* 0.25 ± 0.7 (95%CI: -1.1-0.5) in controls], but these values did not reach statistical significance ($P < 0.12$) (Figure 1).

Symptom association probability

Of the 42 subjects who were evaluated, 35 subjects (83%)

Table 2 Symptom association probability for patients with gastroparesis and controls

MII-pH symptoms	Gastroparesis (*n*)	Controls (*n*)	Positive SAP (*n*)	Negative SAP (*n*)
Chest pain	3	5	1	7
Nausea/ vomiting	2	4	1	6
Regurgitation	3	4	0	14
Hoarseness	0	4	0	6
Heartburn	5	9	0	4
Globus sensation	2	2	0	4
Dysphagia	4	4	1	7
Cough	3	7	1	9
Abdominal pain	7	3	0	10
Belching	6	8	0	14
Bloating	0	2	0	2

SAP: Symptom association probability; MII-pH: Multichannel intraluminal impedance pH.

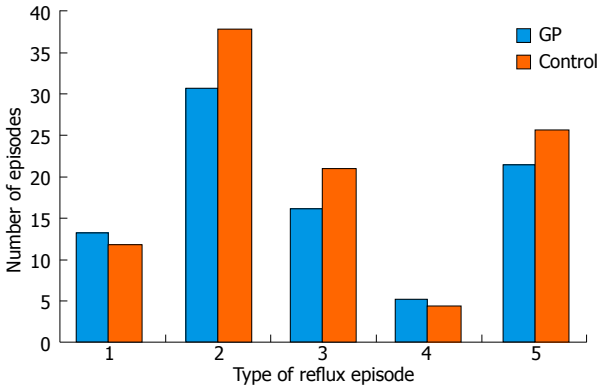

Figure 1 Comparison of the number of reflux episodes for subjects with gastroparesis and controls. 1: Total reflux events; 2: Total acid reflux events; 3: Total non-acid reflux events; 4: Non-acid reflux events pH 4-7; 5: Non-acid reflux events pH > 7. GP: Gastroparesis.

recorded symptoms during the 24-h study period and 7 patients did not have any recorded symptoms. There were 87 total symptoms recorded by the 35 subjects and 33% were typical symptoms and 67% were atypical symptoms of GERD. The GP group accounted for 38% (n = 11) of the total typical symptoms reported and the control group accounted for 62% (n = 18) of typical symptoms. Atypical symptoms of GERD were also more commonly recorded in the control group than the GP group (59% *vs* 41% respectively) (Table 2).

Of the 35 subjects who had recorded symptoms during their MII-pH testing, only 9% (n = 3) had a positive SAP for acid/non-acid reflux and 91% (n = 32) had a negative SAP. Similarly, of the total typical symptoms that were recorded, 7% (n = 2) had a positive SAP and 93% (n = 27) had a negative SAP. Of the 58 atypical symptoms recorded, 3% (n = 2) had a positive SAP and 97% (n = 56) had a negative SAP. Among the 16 subjects with GP, a total of 35 symptoms were recorded and all had a negative SAP. Among the 26 controls, 52 symptoms were recorded, and 8% of those had a positive SAP with the majority (92%) having a negative SAP.

DISCUSSION

Resistance to acid suppression therapy such as PPIs is the most common presentation of GERD in the tertiary care GI practices[16]. A survey of GERD patients receiving PPI therapy shows that 25%-42% of patients are refractory to a once-daily PPI dose, of which only 25% would respond to an increase in PPI dosing to twice daily[17,18]. In addition, 42% of GERD patients surveyed are dissatisfied with their PPI treatment outcomes[19]. GP has long been thought of as a risk factor for refractory GERD due to the impaired gastric accommodation, delayed gastric emptying and the subsequent loss of lower esophageal sphincter tone. Furthermore, as our study shows, symptoms of GP and GERD often overlap as both patients can complain of epigastric pain, abdominal bloating, nausea, and vomiting making it difficult to distinguish between the two disease processes. Given this observed overlap of symptoms, our study aimed to determine whether patients with GP and concurrent symptoms of reflux despite concurrent PPI therapy have an increased frequency and duration of non-acid reflux using impedance pH technology, as compared to those with normal gastric emptying.

Our results indicate that the total number and duration of acid, non-acid, and total reflux events was similar in GP and non-GP cases. While the number of alkaline events was slightly higher in the GP group, these alkaline reflux events represented a small percentage of the total non-acid reflux events observed (20%). Moreover, among the 35 patients who recorded symptoms during the 24 h MII-pH study, only 3 patients had a positive SAP for acid/non-acid reflux. Our findings do not support some prior studies indicating up to 40%-50% of patients with GERD have GP[12,20], and we believe that GP likely accounts for a small percentage of refractory GERD cases than previously evaluated based on conventional pH testing. Our findings based on MII-pH testing indicate that neither acid nor non-acid reflux occur more frequently in patients with GP than those without.

Since only 3 of 35 patients had a positive SAP for acid and non-acid reflux, and given our lack of statistical correlation between GP and acid or non-acid reflux, other causes of refractory GERD need to be explored to explain patients' persistent symptoms. Notably, even the control group with refractory GERD symptoms had a poor symptom correlation with acid and non-acid reflux events. One possible explanation may be the presence of esophageal hypersensitivity, which has been proposed previously as an underlying mechanism in this patient population[16,18,21]. Esophageal distention occurs due to increased reflux volume exposing the esophagus to acidic/non-acid components of the refluxate, which in turn leads to persistent impairment of esophageal mucosa and thus results in esophageal hypersensitivity[21]. In our study, 67% of the reflux events recorded were non-acid with no

correlation to patients' symptoms, making esophageal hypersensitivity a plausible explanation for their continued symptoms.

Our study has some limitations. First, this study was limited to one tertiary care center and the small sample size (n = 42 total with 16 subjects with GP) compromised the overall generalizability of the study, leading to a statistical type Ⅱ error. This may account for our negative findings indicating a lack of difference in acid and non-acid reflux in patients with and without GP. Based on the mean number of acid reflux events in GP and control groups (P = 0.09), the statistical power in the current study is 8%. *Post hoc* power analysis suggests that to achieve 90% statistical power, a sample size of 2592 for each group is necessary. In addition, the GES testing done was not based on the current national standard protocol for obtaining GES and some of the GES studies were not obtained at our institution therefore the accuracy of the measurements may influence our results[22]. This lack of uniformity of GES testing across multiple centers introduces a potential area for error, as patients with or without GP might have been misdiagnosed given lack of standardization. Furthermore, performing the MII-pH study on PPI therapy, as done in our study, may have affected our results in terms of delaying gastric emptying as shown in several studies which could lead to a type Ⅱ error. By delaying acid-dependent peptic activity, PPIs impair hydrolytic digestion and therefore delay gastric emptying[23]. This finding may have clinical implications in the management of GERD in our subjects. Future studies evaluating the effect of GP on acid and non-acid reflux should be done with subjects strictly off PPI therapy 5 d prior to testing. Another limitation is that not all patients were maxed out on PPI therapy prior to the MII-pH study, making continued reflux a potential cause of their continued symptoms. Lastly, GES and MII-pH studies were completed on separate days and in most cases months apart and as a considerable intra-individual variability exists with gastric motility, this may also have contributed to achieving the negative results seen in this study[22].

Our study has several important strengths. This is the first study evaluating whether an association exists between GP and non-acid reflux analyzed by MII-pH monitoring. While GP may have been thought to be associated with refractory GERD, studies have not validated this finding using MII-pH to diagnose acid and non-acid reflux. In addition, the idea that delayed gastric emptying is associated with GERD has been the basis of using prokinetic drugs for the treatment of GERD[20]. Considering our results, it might not be necessary to start these patients on prokinetic agents in addition to acid suppression therapy, unless evidence of esophageal dysmotility is found on esophageal manometry testing, given these drugs have significant side effects and drug-drug interactions. Other medications that improve LES pressure or decrease transient relaxations of the lower esophageal sphincter may be more beneficial for patients with any type of reflux, acid or nonacid, than perhaps improving their gastric emptying. Moreover, our study illustrates that patients with weakly acidic and alkaline reflux likely suffer from esophageal hypersensitivity rather than continued reflux or GP as the main cause of their typical and atypical GERD symptoms. Finally, our study justifies a larger study in which a population of patients with refractory GERD off PPI therapy is evaluated for GP in close proximity to their initial MII-pH analysis combined with manometric esophageal testing. A larger study using the MII-pH as the gold standard for diagnosing non-acid reflux would better delineate whether GP and non-acid reflux are clearly associated.

In conclusion, whether gastroparesis contributes to refractory reflux remains to be established. However, based on our pilot study, a clear relationship does not exist but further studies using larger populations of patients undergoing impedance testing for refractory reflux would help to delineate this relationship.

COMMENTS

Background

Up to 40% of patients with gastroesophageal reflux disease (GERD) have either a partial or incomplete response to proton-pump inhibitors. Gastroparesis has been hypothesized to be a potential cause of refractory GERD in a subset of patients.

Research frontiers

In a case-control study, 42 patients undergoing clinical evaluation for continued heartburn and regurgitation despite acid suppression therapy were evaluated with multi-channel intraluminal impedance pH monitoring and for evidence of gastroparesis.

Innovations and breakthroughs

Their results did not show a difference between acid and non-acid reflux events among patients with gastroparesis and those without the disease process.

Applications

While a clear relationship between gastroparesis and refractory GERD was not shown in their study, the results could be used to conduct further studies using larger populations of patients to help further delineate the relationship.

Terminology

Multi-channel intraluminal impedance pH monitoring is a new technology that can detect intraluminal bolus movement without radiation. When it is combined with pH testing, it can detect both acid and non-acid reflux.

Peer review

The current study is a relevant paper dealing with a difficult to treat and often poorly assessed patient population.

REFERENCES

1 **Camilleri M**, Parkman HP, Shafi MA, Abell TL, Gerson L. Clinical guideline: management of gastroparesis. *Am J Gastroenterol* 2013; **108**: 18-37; quiz 38 [PMID: 23147521 DOI: 10.1038/ajg.2012.373]

2 **Camilleri M**, Grover M, Farrugia G. What are the important subsets of gastroparesis? *Neurogastroenterol Motil* 2012; **24**: 597-603 [PMID: 22626059 DOI: 10.1111/j.1365-2982.2012.01942.x]

3 **Hyett B**, Martinez FJ, Gill BM, Mehra S, Lembo A, Kelly CP, Leffler DA. Delayed radionucleotide gastric emptying studies predict morbidity in diabetics with symptoms of gastroparesis. *Gastroenterology* 2009; **137**: 445-452 [PMID: 19410575 DOI: 10.1053/j.gastro.2009.04.055]

4 **Wang YR**, Fisher RS, Parkman HP. Gastroparesis-related hospitalizations in the United States: trends, characteristics, and

outcomes, 1995-2004. *Am J Gastroenterol* 2008; **103**: 313-322 [PMID: 18047541 DOI: 10.1111/j.1572-0241.2007.01658.x]
5 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187. e1-3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]
6 **Hershcovici T**, Fass R. An algorithm for diagnosis and treatment of refractory GERD. *Best Pract Res Clin Gastroenterol* 2010; **24**: 923-936 [PMID: 21126704 DOI: 10.1016/j.bpg.2010.10.004]
7 **Fass R**. Proton pump inhibitor failure--what are the therapeutic options? *Am J Gastroenterol* 2009; **104** Suppl 2: S33-S38 [PMID: 19262545 DOI: 10.1038/ajg.2009.50]
8 **Hershcovici T**, Fass R. Management of gastroesophageal reflux disease that does not respond well to proton pump inhibitors. *Curr Opin Gastroenterol* 2010; **26**: 367-378 [PMID: 20571388 DOI: 10.1097/MOG.0b013e32833ae2be]
9 **Fass R**. Therapeutic options for refractory gastroesophageal reflux disease. *J Gastroenterol Hepatol* 2012; **27** Suppl 3: 3-7 [PMID: 22486864 DOI: 10.1111/j.1440-1746.2012.07064.x]
10 **Galindo G**, Vassalle J, Marcus SN, Triadafilopoulos G. Multimodality evaluation of patients with gastroesophageal reflux disease symptoms who have failed empiric proton pump inhibitor therapy. *Dis Esophagus* 2013; **26**: 443-450 [PMID: 22862422 DOI: 10.1111/j.1442-2050.2012.01381.x]
11 **Keshavarzian A**, Bushnell DL, Sontag S, Yegelwel EJ, Smid K. Gastric emptying in patients with severe reflux esophagitis. *Am J Gastroenterol* 1991; **86**: 738-742 [PMID: 2038997]
12 **McCallum RW**, Berkowitz DM, Lerner E. Gastric emptying in patients with gastroesophageal reflux. *Gastroenterology* 1981; **80**: 285-291 [PMID: 7450419]
13 **Stacher G**, Lenglinger J, Bergmann H, Schneider C, Hoffmann M, Wölfl G, Stacher-Janotta G. Gastric emptying: a contributory factor in gastro-oesophageal reflux activity? *Gut* 2000; **47**: 661-666 [PMID: 11034582 DOI: 10.1136/gut.47.5.661]
14 **Richter JE**. How to manage refractory GERD. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 658-664 [PMID: 18043675 DOI: 10.1038/ncpgasthep0979]
15 **Weusten BL**, Roelofs JM, Akkermans LM, Van Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. *Gastroenterology* 1994; **107**: 1741-1745 [PMID: 7958686]
16 **Tsoukali E**, Sifrim D. The role of weakly acidic reflux in proton pump inhibitor failure, has dust settled? *J Neurogastroenterol Motil* 2010; **16**: 258-264 [PMID: 20680164 DOI: 10.5056/jnm.2010.16.3.258]
17 **Bardhan KD**. The role of proton pump inhibitors in the treatment of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 1995; **9** Suppl 1: 15-25 [PMID: 7495937 DOI: 10.1111/j.1365-2036.1995.tb00779.x]
18 **Martinez SD**, Malagon IB, Garewal HS, Cui H, Fass R. Non-erosive reflux disease (NERD)--acid reflux and symptom patterns. *Aliment Pharmacol Ther* 2003; **17**: 537-545 [PMID: 12622762 DOI: 10.1046/j.1365-2036.2003.01423.x]
19 **Revicki DA**, Crawley JA, Zodet MW, Levine DS, Joelsson BO. Complete resolution of heartburn symptoms and health-related quality of life in patients with gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 1999; **13**: 1621-1630 [PMID: 10594397 DOI: 10.1046/j.1365-2036.1999.00669.x]
20 **Cunningham KM**, Horowitz M, Riddell PS, Maddern GJ, Myers JC, Holloway RH, Wishart JM, Jamieson GG. Relations among autonomic nerve dysfunction, oesophageal motility, and gastric emptying in gastro-oesophageal reflux disease. *Gut* 1991; **32**: 1436-1440 [PMID: 1773945 DOI: 10.1136/gut.32.12.1436]
21 **Emerenziani S**, Sifrim D, Habib FI, Ribolsi M, Guarino MP, Rizzi M, Caviglia R, Petitti T, Cicala M. Presence of gas in the refluxate enhances reflux perception in non-erosive patients with physiological acid exposure of the oesophagus. *Gut* 2008; **57**: 443-447 [PMID: 17766596 DOI: 10.1136/gut.2007.130104]
22 **Tougas G**, Chen Y, Coates G, Paterson W, Dallaire C, Paré P, Boivin M, Watier A, Daniels S, Diamant N. Standardization of a simplified scintigraphic methodology for the assessment of gastric emptying in a multicenter setting. *Am J Gastroenterol* 2000; **95**: 78-86 [PMID: 10638563 DOI: 10.1111/j.1572-0241.2000.01703.x]
23 **Rasmussen L**, Oster-Jørgensen E, Qvist N, Pedersen SA. The effects of omeprazole on intragastric pH, intestinal motility, and gastric emptying rate. *Scand J Gastroenterol* 1999; **34**: 671-675 [PMID: 10466877 DOI: 10.1080/003655299750025868]

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BRIEF ARTICLE

SEMS *vs* cSEMS in duodenal and small bowel obstruction: High risk of migration in the covered stent group

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Abstract

AIM: To compare clinical success and complications of uncovered self-expanding metal stents (SEMS) *vs* covered SEMS (cSEMS) in obstruction of the small bowel.

METHODS: Technical success, complications and outcome of endoscopic SEMS or cSEMS placement in tumor related obstruction of the duodenum or jejunum were retrospectively assessed. The primary end points were rates of stent migration and overgrowth. Secondary end points were the effect of concomitant biliary drainage on migration rate and overall survival. The data was analyzed according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

RESULTS: Thirty-two SEMS were implanted in 20 patients. In all patients, endoscopic stent implantation was successful. Stent migration was observed in 9 of 16 cSEMS (56%) in comparison to 0/16 SEMS (0%) implantations (P = 0.002). Stent overgrowth did not significantly differ between the two stent types (SEMS: 3/16, 19%; cSEMS: 2/16, 13%). One cSEMS dislodged and had to be recovered from the jejunum by way of laparotomy. Time until migration between SEMS and cSEMS in patients with and without concomitant biliary stents did not significantly differ (HR = 1.530, 95%CI 0.731-6.306; P = 0.556). The mean follow-up was 57 ± 71 d (range: 1-275 d).

CONCLUSION: SEMS and cSEMS placement is safe in small bowel tumor obstruction. However, cSEMS is accompanied with a high rate of migration in comparison to uncovered SEMS.

Key words: Endoscopy; Digestive system; Intestinal neoplasms; Self-expandable metal stents; Tumor obstruction; Self-expandable metal stents complications

Core tip: Gastrointestinal obstruction is a complication of advanced cancer disease. It heavily impacts on patientsi' general condition. Endoscopic implantation of self-expanding metal stents (SEMS) is a safe and established procedure for palliative treatment of tumor obstruction. Covered SEMS are considered favorable concerning reobstruction by inhibiting tumor ingrowth. In contrast, uncovered SEMS might harbor a lower risk of migration and dislocation. In the present study covered SEMS were retrospectively compared with uncovered SEMS in patients with small bowel or duodenal obstruction. Significantly higher migration rates were observed in the covered SEMS group without observing significant increase of the rate of patients with tumor ingrowth indicating that uncovered SEMS should be

favored for palliative treatment of tumor obstruction of the duodenum or the small bowel.

Waidmann O, Trojan J, Friedrich-Rust M, Sarrazin C, Bechstein WO, Ulrich F, Zeuzem S, Albert JG. SEMS *vs* cSEMS in duodenal and small bowel obstruction: High risk of migration in the covered stent group. *World J Gastroenterol* 2013; 19(37): 6199-6206 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6199.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6199

INTRODUCTION

Endoscopic placement of self-expandable metal stents (SEMSs) has become a broadly accepted first line treatment option for patients with advanced malignant intestinal stenosis. Reconstitution of the intestinal transit is paramount in palliation of tumor obstruction, and endoscopic SEMS insertion is weighed against surgical intervention in terms of clinical relief, complication rate, and length of hospital stay of the patient. In the small bowel, duodenal tumor obstruction has increasingly been treated by SEMS placement[1,2], but SEMS insertion in the lower small bowel is less often performed[3,4]. Duodenal SEMS insertion is technically feasible in more than 90% of interventions; it is safe and comes along with a faster start of oral intake, shorter length of hospital stay, lower morbidity and probably reduced in-hospital mortality as compared to surgical treatment[5-13]. SEMS may as well be preferred over surgical treatment in the lower small bowel and at anastomotic small bowel obstructions in case of recurrent malignancy[4].

Tumor in- or over-growth can limit long-term outcome of SEMS in 12% to 21% of cases, but stent occlusion might be reduced by covering the SEMS with silicone or plastic membranes (covered SEMS, cSEMS)[1,2].

Aim of this study was to compare outcome of SEMS *vs* cSEMS in small bowel tumor obstruction and to identify technical feasibility, safety, clinical impact, complications and patient's outcome at follow-up.

MATERIALS AND METHODS

All patients who underwent endoscopic placement of SEMS or cSEMS for small bowel tumor obstruction (duodenum or jejunum) between August 2009 and September 2012 were retrospectively analyzed. Due to advanced or metastatic disease or comorbidity, none of the patients were considered candidates for curative surgical treatment of the tumor. All patients included into this study were symptomatic and were admitted to the hospital because of their obstructive symptoms including nausea, vomiting, bloating, or abdominal pain. At least for some time all patients were suffering from postprandial vomiting. No patient was treated for non-symptomatic stenosis. Indication for jejunal placement of stents was only given in cases of unilocular stenosis by circumscribed peritoneal carcinomatosis. Histology was obtained by endoscopic biopsy from the intestinal tumor or percutaneous needle biopsy from liver metastasis. The therapeutic procedures were ascertained in an interdisciplinary conference with senior physicians from the departments of surgery, gastroenterology and medical oncology and the recommendation to treat by endoscopic stent placement was given in consent.

We retrospectively reviewed the prospectively collected records on technical success of the procedure, clinical benefit, and the incidence of complications including migration and stent occlusion. The patients' outcome at follow-up was additionally registered.

Patients were advised to resume oral intake of liquids within 24 h and to advance to a low-residue diet as tolerated. The status of oral food intake was monitored at one-month intervals on an outpatient basis. In case of recurrence of dysphagia, radiographic imaging (iodine or barium upper gastrointestinal series) and/or upper gastrointestinal endoscopy was performed. Patients who had recurrent symptoms caused by tumor overgrowth were treated by placement of a second intestinal stent.

Endoscopic technique and stent selection

Stents were placed by very experienced gastroenterological endoscopists using a therapeutic gastroscope (GIF-1TQ 160), or a duodenoscope (TJF-Q180V, TJF-160 VR; all Olympus medical Europe, Hamburg, Germany) with a working channel of 3.7 or 4.2 mm, respectively. All stents were inserted through-the-scope (TTS) in combination with over-the-wire technique, and all stents were placed under fluoroscopic guidance. Selection of SEMS *vs* cSEMS was at the appraisal of the endoscopist and cSEMS was preferably chosen in case of advanced tumors with subtotal or complete obstruction intending to avoid later tumor ingrowth. SEMS was preferred over cSEMS in case that the investigator was in fear of blocking biliary outflow by crossing the duodenal papilla by the stent. If complications (migration or overgrowth) occurred, new stents were placed and the stenting procedure in the patient was switched from a covered to an uncovered stent and vice versa. The stents used in this study were self-expandable Nitinol uncovered SEMS (Niti-S, D-Type, TaeWoong medical, South Korea) and covered SEMS [cSEMS; Niti-S pyloric duodenal covered stent (End Bare Type), TaeWoong medical, South Korea]. A stent diameter from 18 to 28 mm and a stent length from 40 to 120 mm were used. The cSEMS provides a silicone covering and has a retrieval suture for preventing tumor-ingrowth and easy removal. It contains a fixed-cell braided structure. The SEMS is a fine mesh tubular prosthesis that facilitates immediate and continuous wall apposition due to the so-called D-weaving technology, *i.e.*, stent cells are unfixed resulting in a high flexibility and retaining its shape even in bending sections of the intestinal tract. In case that a stent did not cover the entire tumor obstruction, two overlapping stents were implanted to bridge the entire obstructed bowel segment and this was accounted

Table 1 Patients' characteristics *n* (%)

Characteristics	SEMS	cSEMS
Stents	16 (50)	16 (50)
Male gender	7 (44)	10 (63)
Age (yr), mean ± SD (range)	70 ± 11 (50-85)	71 ± 11 (50-84)
Localization		
Jejunum, *n*	3	0
Duodenum, *n*	13	16
Disease		
Pancreatic carcinoma, *n*	6	7
Cholangiocellular carcinoma, *n*	3	2
Gallbladder carcinoma, *n*	1	2
Gastric cancer, *n*	3	2
Colorectal cancer, *n*	2	0
Breast cancer metastasis, *n*	1	0
Stenosis due to duodenal ulcer perforation, *n*	0	3
Balloon dilatation of the stent	3 (19)	2 (13)
Concomitant biliary drainage	9 (56)	8 (50)

SEMS: Self-expanding metal stent; cSEMS: Covered SEMS.

as a single application in this study.

Definition

Tumor overgrowth of the stent was defined as deterioration of the patient's condition (recurrence of dysphagia) and detection of narrowing of the stent lumen within or adjacent to the proximal or distal end of the stent mesh as a result of tumor growth, as shown by endoscopic and/or radiologic findings. Tumor ingrowth was defined as tumor obstruction through the stent mesh as a reason for as deterioration of the patient's condition (recurrence of dysphagia). Improvement of vomiting or the intake of fluids or food was assessed qualitatively. Postinterventional complication rate was defined as occurrence of complications within 24 h after stent placement, all other complications were observed until the end of the follow-up period.

Ethics

The retrospective study was approved by the institutional review board (Ethikkomission) of the Johann Wolfgang Goethe-University Hospital.

Statistical analysis

The present study is a retrospective cohort study. The primary endpoints were complications due to stent implantation including tumor overgrowth and stent migration. The secondary end points were effect of concomitant biliary stenting on migration rates and overall survival. Statistical analyses were performed with SPSS (Version 22.0, IBM, NY, United States). Predictors of survival were determined using a univariate Cox regression hazard model. Death was recorded as event. Survival curves with the estimated hazards where calculated with the Cox regression model. The patients at risk at the individual time points are shown in the figures. The data was analyzed according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines[14].

Table 2 Complications *n* (%)

Complications	SEMS	cSEMS	*P* value
Duration of procedure (min), median (range)	60 (40-121)	60 (31-160)	0.867
Migration	0 (0)	9 (56)	0.002
Time until migration (d), mean ± SD (range)	-	30 ± 52 (1-161)	NA
Tumor overgrowth	3 (19)	2 (13)	0.725
Tumor ingrowth, *n*	0	0	NA
Time until tumor overgrowth (d), mean ± SD (range)	143 ± 95 (39-224)	96 ± 105 (22-170)	0.572
Overall survival (d), median, range	40 (3-275)	75 (11-426)	0.431

NA: Not available; SEMS: Self-expanding metal stent; cSEMS: Covered SEMS.

RESULTS

Thirty-two cases of stent insertion were included in this study: 16 cSEMSs and 16 SEMSs were placed in 20 patients. Patient characteristics are shown in Table 1. Five patients received two overlapping stents; in the remaining 27 interventions a singular stent was put in place. Whereas all covered SEMS were implanted in the duodenum, 3 of 16 uncovered SEMS were inserted into the jejunum. The main etiology of gastric outlet obstruction was pancreatic cancer, followed by cholangiocellular carcinoma or gallbladder carcinoma (Table 1). All three jejunal SEMS were placed due to an obstruction that was caused by a circumscribed manifestation of peritoneal carcinomatosis in gastric cancer patients. Nine of the SEMS (56%) and eight of the cSEMS (50%) were placed in patients who presented with concomitant biliary tract stenosis; all these patients were treated with placement of plastic stents or SEMS/cSEMS into the common bile duct (CBD), and plastic endoprosthesis were replaced by biliary SEMS at the time of duodenal SEMS insertion in all patients.

We observed technical success of SEMS placement in all cases without occurrence of peri-interventional complications. In three (SEMS) and two (cSEMS) stent placements, respectively, balloon dilatation was needed for complete expansion of the stents. The duration of endoscopic procedure did not significantly differ between SEMS and cSEMS (Table 2). The clinical condition ameliorated in 14 of 16 (87.5%) cases treated with SEMS and intake of fluids or food improved. In contrast, in patients treated with cSEMS the clinical condition improved in 12 of 16 (75%) cases only. However, this difference was not statistically significant ($P = 0.564$).

The mean follow-up time ± SD was 57 ± 71 d with a range of 1-275 d. In patients with gastric outlet obstruction and concomitant biliary obstruction no migration or occlusion of the bile duct stents was observed. Nine of the 16 cSEMS (56%) migrated within the observation time. In one of the patients the dislodged stent had to be recovered from the jejunum by laparotomy. The patient

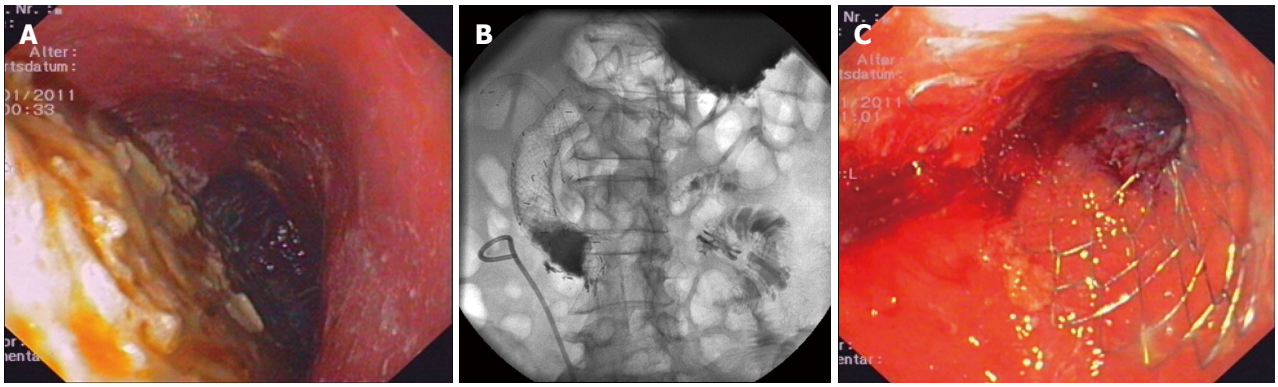

Figure 1 Self-expanding metal stents insertion in duodenal tumor obstruction. A: Retention of secretions and food in the stomach; B: Duodenal self-expanding metal stents (SEMS) together with biliary SEMS in X-ray; C: SEMS in endoscopic imaging.

was dismissed from hospital treatment after recovery from the surgery. On the contrary none of the SEMS dislocated. SEMS placement was superior to cSEMS for duodenal location of obstructions concerning migration rate (0% *vs* 56%). However, although none of the three SEMS placed in the jejunum migrated within time no further conclusion can be drawn for a superiority of SEMS in the jejunal location as no cSEMS was placed in the jejunum. The mean time until migration ± SD was 30 ± 52 d with a range of 1-161 d.

As stents in situ of the CBD might give hold to the duodenal SEMS/cSEMS and might be associated with a reduced rate of stent migration, we analyzed the group of patients with CBD SEMS/cSEMS and concomitant duodenal SEMS/cSEMS placement separately. In 17 of 32 cases, a combined biliary and duodenal SEMS/cSEMS insertion had been undertaken. Comparing the time until migration of stents in patients with and without biliary SEMS/cSEMS, there was no difference in migration of duodenal stents (HR = 1.530, 95%CI: 0.731-6.306; P = 0.556). In those 17 patients in whom a biliary SEMS was in place, all six events of stent migration were observed in patients with duodenal cSEMS, whereas no migration was seen in patients with uncovered duodenal SEMS (P = 0.008). A representative example of duodenal stent with concomitant biliary metal stent implantation is shown in Figure 1.

Overgrowth of a SEMS by progressive cancer occurred in three cases, whereas this complication was seen in two of the cSEMS patients. There was no tumor ingrowth into any of the stents (SEMS or cSEMS) observed. In case of tumor overgrowth or migration, a new stent was placed into the stenosis and migrated cSEMS were replaced by uncovered SEMS. A flow chart demonstrating the algorithm of stent treatment is displayed in Figure 2.

Thirteen patients died within the observation time. The median overall survival was 74 d (Figure 3A). To assess the influence of the stent type on survival, overall survival times for the two kinds of stents were compared. There was no significant difference between SEMS and cSEMS concerning overall survival according to a univariate Cox regression model (Figure 3B).

DISCUSSION

In this comparative study, we observed a high rate (> 50%) of stent migration in the covered SEMS group in comparison to the non-covered SEMS group (0%) in palliation of duodenal or small bowel obstruction. Concurrently, technical feasibility of stent placement (TTS technique) was similar, and relief of symptoms was equal in both patient groups. Insertion of SEMS was as safe as cSEMS implantation during periinterventional surveillance of the patient. Thereby, biliary SEMS did not prevent migration of duodenal cSEMS migration, and the migration rate in patients with and without concomitant biliary stents was similar. We did not observe clinically significant tumor ingrowth in the SEMS or cSEMS group. However, the overall survival times were quite short and in patients with longer survival times cSEMS might show advantages concerning tumor ingrowth rates.

Randomized trials comparing SEMS *vs* cSEMS treatment in the small bowel has not been reported up to date, but an increased risk of stent migration in covered SEMS has been reported in colonic tumor palliation: Stent migration was four times as common in the covered SEMS group as in the non-covered stent group in a recent meta-analysis[15]. In patients with inoperable gastric cancer, comparison of cSEMS *vs* SEMS yielded similar results: Migration was observed in 26.0% of patients, in comparison to 2.8% in non-covered SEMS in a randomized trial[16]. Our results suggest that migration rate in the small bowel is even higher than in the stomach or large bowel. Technical success rate and clinical success was similar in cSEMS *vs* SEMS in both studies, and immediate complications were near to zero. Migration rates are low (1%-3% of cases) in other studies reporting on SEMS insertion in the duodenum and small bowel[1,17].

As the duration of the endoscopic treatments and the responsible endoscopists did not differ between the two cohorts, we consider two factors to contribute mainly on migration in cSEMS *vs* SEMS study: First, the stent cover minimizes hyperplastic tissue to get hold of the stent

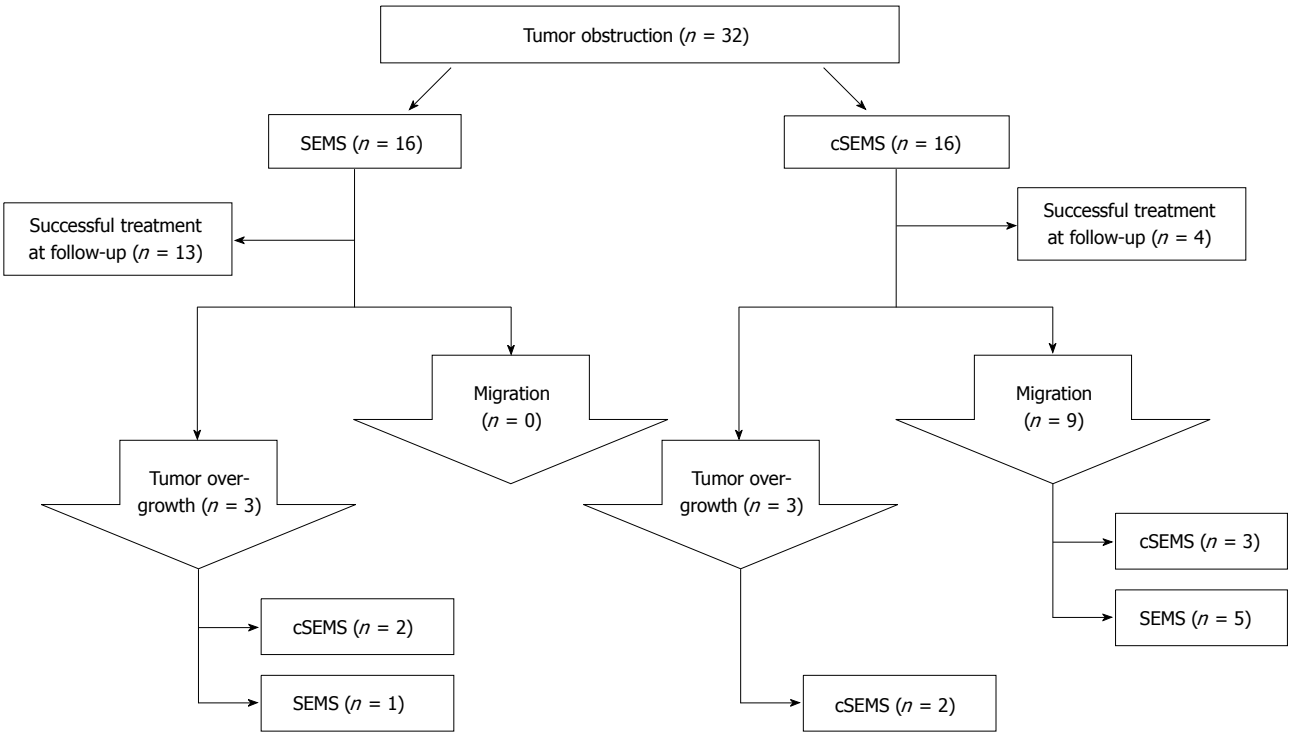

Figure 2 The treatment algorithm for self-expanding metal stents placement. SEMS: Self-expanding metal stents; cSEMS: Covered SEMS.

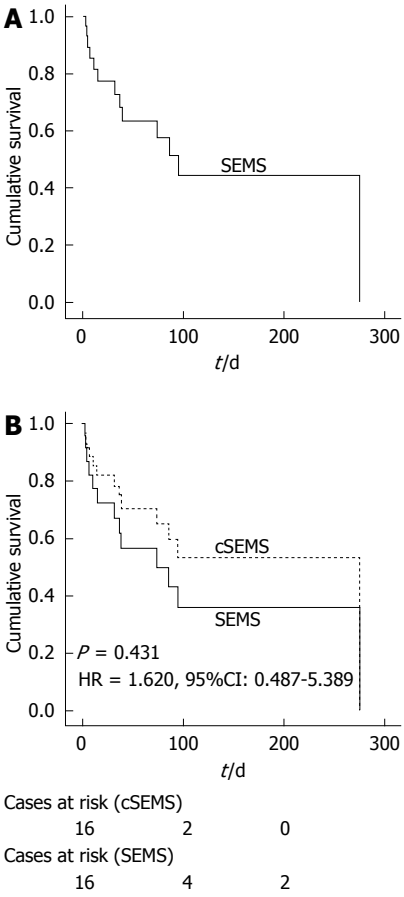

Figure 3 Survival curves. A: The overall survival curve calculated with the Cox regression model; B: Survival curves for self-expanding metal stents (SEMS) and covered SEMS (cSEMS) placements calculated with the Cox regression model.

mesh and partial ingrowth of tumorous and hyperplastic tissue is prevented, thus minimizing any anti-migration effect that the stent may provide against the natural motility of the small bowel. Moreover, we think that the fixed-cell braided structure of the SEMS we used might have significantly contributed to migration by causing the stent to straighten itself by use of its axial expansion force. In opposite, the so-called D-weaving technology of the SEMS with unfixed stent cells might have resulted in a higher flexibility and retention of its shape even at bending sections of the intestinal tract, such as the duodenum. The motility of the small bowel contributes to the progression of the stent. Stent migration can be reduced by endoscopic clip fixation of stents in the duodenal position[18]. This procedure might be impracticable and only short-lasting, though, as clips usually dislodge in the short term.

The use of SEMS in duodenal obstruction might be compromised by concomitant biliary obstruction. However, combined endoscopic treatment is safe and successful in the majority of cases with only minor complications (0%-16% of cases)[19-23] even if others report found much higher incidence of complications (up to 52%)[24]. In our cohort of patients there were no treatment related complications of biliary drainage by duodenal stents. Migration rates were also not affected by the existence of biliary stenting. However, all events of migration of duodenal stents in patients receiving combined biliary and duodenal stenting happened in patients who had obtained cSEMS. Therefore, biliary stenting may protect from duodenal stent migration, if the biliary stents are placed in between the meshes of uncovered SEMS. The

Table 3 Complication rates in small bowel self-expanding metal stents placement in retrospective analyses

Study	Patients, stents	Site of tumor obstruction	Tumor overgrowth	Migration	Bleeding	Perforation
Costamagna *et al*[1]	202, 212	Endoscopic duodenal stenting	12.4%	1.5%	3.0%	0.5%
van Hooft *et al*[2]	50, 57	Endoscopic stenting for gastric outlet obstruction	21.0%	4.0%	0.0%	0.0%
Jeong *et al*[4]	25, 28	Gastrojejunostomy, esophagojejunostomy, cSEMS	17.0%	4.0%	0.0%	1.0%
Chandrasegaram *et al*[7]	26, 31	Endoscopic stenting *vs* operative gastrojejunostomy	12.0%	0.0%	0.0%	0.0%
Jang *et al*[17]	583, 603	Peripyloric region, nonperipyloric region, duodenum alone anastomosis (Billroth Ⅰ, Billroth Ⅱ), jejunum	3.8%	NM	NM	< 1.0%
Kim *et al*[25]	50, 50	Endoscopic stenting for malignant gastroduodenal obstructions	18.0%	10.0%	0.0%	0.0%
Wong *et al*[26]	6, 6	Surgical *vs* endoscopic palliation	NM	NM	NM	NM
Mosler *et al*[27]	36, 52	Endoscopic stenting of nonesophageal upper gastrointestinal stenosis	11.0%	14.0%	0.0%	6.0%
Kim *et al*[28]	213, 236	Malignant gastroduodenal obstruction	7.0%	4.0%	1.0%	0.0%
Bang *et al*[29]	134, 132	Endoscopic treatment for malignant antropyloric and duodenal	cSEMS 5.7% SEMS 19.0%	cSEMS 26.4% SEMS 6.3%	2.2%	< 1.0%
Keränen *et al*[30]	104, 130	Endoscopic treatment for malignant gastric outlet obstruction	18.0%	0.0%	0.0%	1.9%
Ahn *et al*[31]	47, 52	Malignant gastroduodenal obstruction, uncovered SEMS	11.0%	2.0%	0.0%	4.0%
Canena *et al*[32]	74, 80	Endoscopic stenting for gastric outlet obstruction	9.5%	0.0%	1.4%	0.0%
Cha *et al*[33]	85, 85	Endoscopic stenting for gastroduodenal obstruction	29.0%	4.0%	4.0%	4.0%
Own data	20, 32	Small bowel/duodenum	cSEMS 13.0% SEMS 19.0%	cSEMS 56.0% SEMS 0.0%	0.0%	0.0%

SEMS: Self-expanding metal stent; cSEMS: Covered SEMS; NM: Not mentioned.

advantage of SEMS in comparison to cSEMS might be lower rates of tumor occlusion[1,2,19]. In contrast in patients receiving cSEMS recurrence of tumor occlusion is rarely observed[19]. The rate of stent occlusion did not significantly differ between patients with SEMS (19%) and cSEMS (13%) in our study, though. Also in another report no differences were found between SEMS and SEMS concerning necessity of re-interventions[25]. An overview of literature concerning complications of SEMS placements is provided in Table 3.

The limitation of the current study is the retrospective non-randomized approach. However, in conclusion, technical feasibility, tumor overgrowth, and overall survival of the patients are comparable in SEMS *vs* cSEMS, but migration rate is much higher in cSEMS as migration was observed in none of the SEMS group patients.

We prefer SEMS over cSEMS insertion as first choice for malignant duodenal and small bowel obstruction and restrict use of cSEMS in cases with fast tumor ingrowth. Prospective randomized trials are needed to compare SEMS and cSEMS for small bowel obstruction.

COMMENTS

Background

Duodenal and small bowel obstructions are complications of advanced cancer disease. Endoscopic implantation of nitinol based self-expanding metal stents (SEMS) is a safe and established procedure for palliative treatment of tumor obstruction. Covered and uncovered SEMS differ in their characteristics concerning risk of tumor ingrowth and overgrowth or migration and dislocation. But up to now, there is no data provided that covered or uncovered SEMS should be favored for treatment of tumor stenosis in the duodenum or small bowel.

Research frontiers

Endoscopic treatment of duodenal or small bowel obstruction is an established treatment procedure for malignant stenosis. However, no thorough analysis comparing covered SEMS and uncovered SEMS in for tumor related stenosis has been reported. The authors hypothesized, that covered SEMS and uncovered SEMS differ concerning complications in the indicated localization.

Innovations and breakthroughs

The authors learnt that covered SEMS showed significant higher migrations rates than uncovered SEMS when placed in the duodenal position. In contrast, no significant differences concerning re-obstruction of the lumen or overall survival after SEMS implantation were found.

Applications

The authors conclude that uncovered SEMS should be preferred when SEMS implantation in the duodenum is performed.

Terminology

SEMS are self-expanding metal stents which are often made of nitinol alloyings and are placed by guide wired and under fluoroscopic control in endoscopic procedures. SEMS can be used uncovered or covered with silicone or other materials.

Peer review

The authors showed in their retrospective analysis for the first time that uncovered SEMS might be preferred for malignant duodenal or small stenosis, as covered SEMS show much higher rates of migration. The results may help to reduce complications raised by migrated SEMS.

REFERENCES

1 **Costamagna G**, Tringali A, Spicak J, Mutignani M, Shaw J, Roy A, Johnsson E, De Moura EG, Cheng S, Ponchon T, Bittinger M, Messmann H, Neuhaus H, Schumacher B, Laugier R, Saarnio J, Ariqueta FI. Treatment of malignant gastroduodenal obstruction with a nitinol self-expanding metal stent: an international prospective multicentre registry. *Dig Liver Dis* 2012; **44**: 37-43 [PMID: 21937292 DOI: 10.1016/j.dld.2011.08.012]

2 **van Hooft JE**, van Montfoort ML, Jeurnink SM, Bruno MJ, Dijkgraaf MG, Siersema PD, Fockens P. Safety and efficacy of a new non-foreshortening nitinol stent in malignant gastric outlet obstruction (DUONITI study): a prospective, multicenter study. *Endoscopy* 2011; **43**: 671-675 [PMID: 21656455 DOI: 10.1055/s-0030-1256383]

3 **Lee H**, Park JC, Shin SK, Lee SK, Lee YC. Preliminary study of enteroscopy-guided, self-expandable metal stent placement for malignant small bowel obstruction. *J Gastroenterol Hepatol* 2012; **27**: 1181-1186 [PMID: 22414138 DOI: 10.1111/j.1440-1746.2012.07113.x]

4 **Jeong JY**, Kim YJ, Han JK, Lee JM, Lee KH, Choi BI, Yang HK, Lee KU. Palliation of anastomotic obstructions in recurrent gastric carcinoma with the use of covered metallic stents: clinical results in 25 patients. *Surgery* 2004; **135**: 171-177 [PMID: 14739852]

5 **Hosono S**, Ohtani H, Arimoto Y, Kanamiya Y. Endoscopic stenting versus surgical gastroenterostomy for palliation of malignant gastroduodenal obstruction: a meta-analysis. *J Gastroenterol* 2007; **42**: 283-290 [PMID: 17464457]

6 **Jeurnink SM**, Polinder S, Steyerberg EW, Kuipers EJ, Siersema PD. Cost comparison of gastrojejunostomy versus duodenal stent placement for malignant gastric outlet obstruction. *J Gastroenterol* 2010; **45**: 537-543 [PMID: 20033227 DOI: 10.1007/s00535-009-0181-0]

7 **Chandrasegaram MD**, Eslick GD, Mansfield CO, Liem H, Richardson M, Ahmed S, Cox MR. Endoscopic stenting versus operative gastrojejunostomy for malignant gastric outlet obstruction. *Surg Endosc* 2012; **26**: 323-329 [PMID: 21898024 DOI: 10.1007/s00464-011-1870-3]

8 **Dormann A**, Meisner S, Verin N, Wenk Lang A. Self-expanding metal stents for gastroduodenal malignancies: systematic review of their clinical effectiveness. *Endoscopy* 2004; **36**: 543-550 [PMID: 15202052]

9 **Fukami N**, Anderson MA, Khan K, Harrison ME, Appalaneni V, Ben-Menachem T, Decker GA, Fanelli RD, Fisher L, Ikenberry SO, Jain R, Jue TL, Krinsky ML, Maple JT, Sharaf RN, Dominitz JA. The role of endoscopy in gastroduodenal obstruction and gastroparesis. *Gastrointest Endosc* 2011; **74**: 13-21 [PMID: 21704805 DOI: 10.1016/j.gie.2010.12.003]

10 **Varadarajulu S**, Banerjee S, Barth B, Desilets D, Kaul V, Kethu S, Pedrosa M, Pfau P, Tokar J, Wang A, Song LM, Rodriguez S. Enteral stents. *Gastrointest Endosc* 2011; **74**: 455-464 [PMID: 21762904 DOI: 10.1016/j.gie.2011.04.011]

11 **Piesman M**, Kozarek RA, Brandabur JJ, Pleskow DK, Chuttani R, Eysselein VE, Silverman WB, Vargo JJ, Waxman I, Catalano MF, Baron TH, Parsons WG, Slivka A, Carr-Locke DL. Improved oral intake after palliative duodenal stenting for malignant obstruction: a prospective multicenter clinical trial. *Am J Gastroenterol* 2009; **104**: 2404-2411 [PMID: 19707192 DOI: 10.1038/ajg.2009.409]

12 **van Hooft JE**, Uitdehaag MJ, Bruno MJ, Timmer R, Siersema PD, Dijkgraaf MG, Fockens P. Efficacy and safety of the new WallFlex enteral stent in palliative treatment of malignant gastric outlet obstruction (DUOFLEX study): a prospective multicenter study. *Gastrointest Endosc* 2009; **69**: 1059-1066 [PMID: 19152912 DOI: 10.1016/j.gie.2008.07.026]

13 **Vlavianos P**, Zabron A. Clinical outcomes, quality of life, advantages and disadvantages of metal stent placement in the upper gastrointestinal tract. *Curr Opin Support Palliat Care* 2012; **6**: 27-32 [PMID: 22228029 DOI: 10.1097/SPC.0b013e32834f6004]

14 **von Elm E**, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; **147**: 573-577 [PMID: 17938396]

15 **Zhang Y**, Shi J, Shi B, Song CY, Xie WF, Chen YX. Comparison of efficacy between uncovered and covered self-expanding metallic stents in malignant large bowel obstruction: a systematic review and meta-analysis. *Colorectal Dis* 2012; **14**: e367-e374 [PMID: 22540666 DOI: 10.1111/j.1463-1318.2012.03056.x]

16 **Kim CG**, Choi IJ, Lee JY, Cho SJ, Park SR, Lee JH, Ryu KW, Kim YW, Park YI. Covered versus uncovered self-expandable metallic stents for palliation of malignant pyloric obstruction in gastric cancer patients: a randomized, prospective study. *Gastrointest Endosc* 2010; **72**: 25-32 [PMID: 20381802 DOI: 10.1016/j.gie.2010.01.039]

17 **Jang JK**, Song HY, Kim JH, Song M, Park JH, Kim EY. Tumor overgrowth after expandable metallic stent placement: experience in 583 patients with malignant gastroduodenal obstruction. *AJR Am J Roentgenol* 2011; **196**: W831-W836 [PMID: 21606277 DOI: 10.2214/AJR.10.5861]

18 **Kim ID**, Kang DH, Choi CW, Kim HW, Jung WJ, Lee DH, Chung CW, Yoo JJ, Ryu JH. Prevention of covered enteral stent migration in patients with malignant gastric outlet obstruction: a pilot study of anchoring with endoscopic clips. *Scand J Gastroenterol* 2010; **45**: 100-105 [PMID: 20030581 DOI: 10.3109/00365520903410554]

19 **Katsanos K**, Sabharwal T, Adam A. Stenting of the upper gastrointestinal tract: current status. *Cardiovasc Intervent Radiol* 2010; **33**: 690-705 [PMID: 20521050 DOI: 10.1007/s00270-010-9862-6]

20 **Baron TH**. Management of simultaneous biliary and duodenal obstruction: the endoscopic perspective. *Gut Liver* 2010; **4** Suppl 1: S50-S56 [PMID: 21103295 DOI: 10.5009/gnl.2010.4.S1.S50]

21 **Kim KO**, Kim TN, Lee HC. Effectiveness of combined biliary and duodenal stenting in patients with malignant biliary and duodenal obstruction. *Scand J Gastroenterol* 2012; **47**: 962-967 [PMID: 22571283 DOI: 10.3109/00365521.2012.677956]

22 **Moon JH**, Choi HJ, Ko BM, Koo HC, Hong SJ, Cheon YK, Cho YD, Lee MS, Shim CS. Combined endoscopic stent-in-stent placement for malignant biliary and duodenal obstruction by using a new duodenal metal stent (with videos). *Gastrointest Endosc* 2009; **70**: 772-777 [PMID: 19595319 DOI: 10.1016/j.gie.2009.04.013]

23 **Maire F**, Hammel P, Ponsot P, Aubert A, O'Toole D, Hentic O, Levy P, Ruszniewski P. Long-term outcome of biliary and duodenal stents in palliative treatment of patients with unresectable adenocarcinoma of the head of pancreas. *Am J Gastroenterol* 2006; **101**: 735-742 [PMID: 16635221]

24 **Hamada T**, Nakai Y, Isayama H, Sasaki T, Kogure H, Kawakubo K, Sasahira N, Yamamoto N, Togawa O, Mizuno S, Ito Y, Hirano K, Toda N, Tada M, Koike K. Duodenal metal stent placement is a risk factor for biliary metal stent dysfunction: an analysis using a time-dependent covariate. *Surg Endosc* 2013; **27**: 1243-1248 [PMID: 23073685]

25 **Kim YW**, Choi CW, Kang DH, Kim HW, Chung CU, Kim DU, Park SB, Park KT, Kim S, Jeung EJ, Bae YM. A double-layered (comvi) self-expandable metal stent for malignant gastroduodenal obstruction: a prospective multicenter study. *Dig Dis Sci* 2011; **56**: 2030-2036 [PMID: 21264512 DOI: 10.1007/s10620-011-1566-5]

26 **Wong YT**, Brams DM, Munson L, Sanders L, Heiss F, Chase M, Birkett DH. Gastric outlet obstruction secondary to pancreatic cancer: surgical vs endoscopic palliation. *Surg Endosc* 2002; **16**: 310-312 [PMID: 11967685]

27 **Mosler P**, Mergener KD, Brandabur JJ, Schembre DB, Kozarek RA. Palliation of gastric outlet obstruction and proximal small bowel obstruction with self-expandable metal stents: a single center series. *J Clin Gastroenterol* 2005; **39**: 124-128 [PMID: 15681907]

28 **Kim JH**, Song HY, Shin JH, Choi E, Kim TW, Jung HY, Lee GH, Lee SK, Kim MH, Ryu MH, Kang YK, Kim BS, Yook JH. Metallic stent placement in the palliative treatment of malignant gastroduodenal obstructions: prospective evaluation of results and factors influencing outcome in 213 patients. *Gastrointest Endosc* 2007; **66**: 256-264 [PMID: 17643698]

29 **Bang S**, Kim HJ, Park JY, Park YS, Kim MH, Park SW, Lee' YC, Song SY. Effectiveness of self-expanding metal stents for malignant antropyloric and duodenal obstruction with a comparison between covered and uncovered stents. *Hepatogastroenterology* 2008; **55**: 2091-2095 [PMID: 19260483]

30 **Keränen I**, Udd M, Lepistö A, Halttunen J, Kylänpää L. Outcome for self-expandable metal stents in malignant gastroduodenal obstruction: single-center experience with 104 patients. *Surg Endosc* 2009 Sep 3; Epub ahead of print [PMID: 19730943]
31 **Ahn HS**, Hong SJ, Moon JH, Ko BM, Choi HJ, Han JP, Park JS, Kang MS, Cho JY, Lee JS, Lee MS. Uncovered self-expandable metallic stent placement as a first-line palliative therapy in unresectable malignant duodenal obstruction. *J Dig Dis* 2012; **13**: 628-633 [PMID: 23134154 DOI: 10.1111/j.1751-2980.2012.00644.x]
32 **Canena JM**, Lagos AC, Marques IN, Patrocínio SD, Tomé MG, Liberato MA, Romão CM, Coutinho AP, Veiga PM, Neves BC, Além HD, Gonçalves JA. Oral intake throughout the patients' lives after palliative metallic stent placement for malignant gastroduodenal obstruction: a retrospective multicentre study. *Eur J Gastroenterol Hepatol* 2012; **24**: 747-755 [PMID: 22522142 DOI: 10.1097/MEG.0b013e328353d9d3]
33 **Cha BH**, Lee SH, Kim JE, Yoo JY, Park YS, Kim JW, Jeong SH, Kim N, Lee DH, Hwang JH. Endoscopic self-expandable metallic stent placement in malignant pyloric or duodenal obstruction: does chemotherapy affect stent patency? *Asia Pac J Clin Oncol* 2013; **9**: 162-168 [PMID: 23057590 DOI: 10.1111/j.1743-7563.2012.01590.x]

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BRIEF ARTICLE

Inflammatory bowel disease serology in Asia and the West

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Abstract

AIM: To study serological antibodies in Caucasians and Asians, in health and inflammatory bowel disease (IBD), in Australia and Hong Kong (HK).

METHODS: Anti-glycan antibodies [anti-chitobioside (ACCA), anti-laminaribioside (ALCA)], and anti-mannobioside (AMCA), anti-*Saccharomyces cervisiae* (gASCA); and atypical perinuclear anti-neutrophil cytoplasmic antibody (pANCA) were tested in IBD patients, their unaffected relatives, and healthy controls in Australia and HK (China). Antibody status (positive or negative) and titre was compared between subjects of different geography, ethnicity and disease state.

RESULTS: Ninety subjects were evaluated: 21 Crohn's disease (CD), 32 ulcerative colitis (UC), 29 healthy controls, and 8 IBD patient relatives. Forty eight subjects were Australian (29 Caucasian and 19 ethnic Han Chinese) and 42 were from HK (all Han Chinese). Caucasian CD patients had a significantly higher antibody prevalence of gASCA (67% *vs* 3%, $P < 0.001$), ALCA (44% *vs* 6%, $P = 0.005$), and AMCA (67% *vs* 15%, $P = 0.002$), whereas HK CD patients had a higher prevalence of only AMCA (58% *vs* 25%, $P = 0.035$), when compared with UC and healthy subjects in both countries. Caucasian CD had significantly higher gASCA prevalence (67% *vs* 0%, $P < 0.001$) and titre (median 59 *vs* 9, $P = 0.002$) than HK CD patients. Prevalence and titres of ALCA, ACCA and AMCA did not differ between CD in the two countries. Presence of at least one antibody was higher in Caucasian than HK CD patients (100% *vs* 58%, $P = 0.045$). pANCA did not differ between countries or ethnicity.

CONCLUSION: Serologic CD responses differ between HK Asian and Australian Caucasian patients. Different genetic, environmental or disease pathogenic factors may account for these differences.

Key words: Crohn's disease; Ulcerative colitis; Serological antibodies; Asia; Ethnic; Anti-*Saccharomyces cervisiae* antibodies; Anti-chitobioside antibodies; Anti-laminaribioside antibodies; Anti-mannobioside antibodies; Atypical perinuclear anti-neutrophil cytoplasmic antibodies

Core tip: Serological antibodies to enteric antigens are a hallmark of inflammatory bowel disease (IBD) and may carry pathogenic and prognostic significance. There is limited information about their role and prevalence in Asian patients. We evaluated anti-glycan antibodies (anti-chitobioside, anti-laminaribioside, and anti-mannobioside), anti-*Saccharomyces cervisiae*; and atypical

perinuclear anti-neutrophil cytoplasmic antibody in IBD patients, their unaffected relatives, and healthy controls in Australia and Hong Kong (China). Serologic responses were found to differ between Asian and Caucasian patients. Different genetic, environmental or disease pathogenic factors may account for these differences.

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are related to a mucosal immune response to antigenic stimulation from the gut microbiota on a background of genetic susceptibility[1]. Serological antibodies for inflammatory bowel disease (IBD) have a role as diagnostic markers for IBD and assist in disease stratification[2].

Glycans are carbohydrate surface components, which can be found on immune cells, erythrocytes, tissue matrices and microorganisms. They likely reflect the interaction between the immune system and glycosylated cell wall components of microbiota such as fungi, yeast, and bacteria[3]. Anti-*Saccharomyces cerevisiae* (gASCA) (IgA and IgG) antibodies are directed against the cell wall mannan of the yeast *Saccharomyces* that shares homology with intestinal bacteria[4]. gASCA (antibodies against covalently immobilized mannan)[5] have been found to be comparable to "conventional" ASCA[6]. Anti-laminaribioside carbohydrate IgG antibodies (ALCA), anti-chitobioside carbohydrate IgA antibodies (ACCA), anti-mannobioside carbohydrate IgG antibodies (AMCA) were first reported in 2006[5] and discovered using GlycoChip glycan array technology[7]. These antibodies may allow differentiation of IBD from health, define between IBD subtypes, and have been associated with a more complicated CD behaviour[2,5]. Atypical perinuclear anti-neutrophil cytoplasmic antibody (pANCA) is regarded as a marker of UC, as it has a higher prevalence in UC than in CD or healthy controls[8].

Until two decades ago IBD was rare in Asia[9], but recent population-based and referral centre cohorts[10,11] have shown a rising incidence and prevalence of IBD in Asia[12]. These temporal trends in disease incidence and prevalence may provide insights into possible etiologic factors, such as genetic *vs* environmental. As serologic antibodies may represent an interface between a patient's genetic make-up and their environment, we hypothesised that evaluation of serologic responses in areas of increasing incidence may provide an insight into these complex interactions. Most data on serological antibodies are derived from North American or European cohorts. There are no publications of the prevalence of the anti-glycan antibodies in Asian cohorts, either in Asia or in Asians abroad.

This study aimed to provide an initial insight into the prevalence and magnitude of the anti-glycan antibodies, and pANCA in IBD, compared to control groups, in Han Chinese (referred to as Asian) and Caucasian subjects in Australia and in Han Chinese subjects in Hong Kong (China).

MATERIALS AND METHODS

Patient population

Serum samples were obtained from consented consecutive subjects, regardless of disease extent or duration, from IBD centres in Melbourne, Australia and Hong Kong (China).

IBD diagnosis and differentiation into UC and CD was made based on accepted clinical, endoscopic, histopathological, and radiological findings. Patient characteristics are shown in Table 1. The healthy subjects consisted of patients undergoing a colonoscopy for a family history of cancer or polyps, with a subsequent normal colonoscopy. Eight first degree relatives of IBD subjects (2 of UC, 6 of CD) who were undergoing a colonoscopy for cancer screening were also studied. Signed informed consent was obtained from all participants. The study was approved by the Ethics Committees of St Vincent's Public and Private Hospitals Melbourne, and The Chinese University of Hong Kong.

Serological analysis

After blood was taken, serum was immediately separated by centrifugation and then frozen at -80 ℃ until use. All sera were processed anonymously.

The IBDX ELISA kit was used to detect gASCA IgG, ALCA IgG, ACCA IgA, AMCA IgG, following the manufacturer's recommendations (Glycominds Ltd, Lod, Israel). The cutoff values were those supplied by the manufacturer: 50, 90, 60, 100 EUs for gASCA IgG, ACCA, ALCA, and AMCA, respectively. pANCA was performed using indirect immunofluorescence on ethanol and formalin-fixed neutrophil substrate slides.

For the titre of immune response of the anti-glycan antibodies, quartile scores for each serologic antibody were calculated, as described previously[6,13,14]. For each patient each antibody titre was assigned to a quartile score of 1 (lowest), 2, 3, or 4 (highest). By adding individual quartile scores for each glycan antigen a semi-quantitative quartile sum score (QSS) (range 4-16), representing the cumulative quantitative immune response toward all four antigens for each patient, was obtained.

Statistical analysis

Using the suggested cut-off values for each antibody, positive or negative status was determined for each subject. In addition, antibody titres were divided into four groups based on the quartiles (see description above). Discrete parameters were assessed as percentages and

Table 1 Subject demographics and disease characteristics *n* (%)

Country	Group	Ethnicity	Group No.	Age (yr) mean ± SD	Female	Never smoker	Family history of IBD	CD (severe behaviour)	CD (ileocolonic location)	UC proctitis
Australia	Crohn's	Caucasian	9	29 ± 12	4 (44)	3 (33)	0 (0)	8 (89)	7 (78)	-
(*n* = 48)	UC	Caucasian	10	37 ± 11	5 (50)	7 (70)	1 (10)	-	-	1 (10)
		Asian	10	45 ± 14	2 (20)	8 (80)	0 (0)	-	-	3 (10)
	Healthy	Caucasian	10	46 ± 12	5 (50)	4 (40)	0 (0)	-	-	-
		Asian	9	51 ± 11	4 (44)	7 (78)	1 (11)	-	-	-
Hong Kong	Crohn's	Asian	12	38 ± 15	7 (58)	7 (58)	1 (8)	3 (25)	9 (75)	-
(*n* = 42)	UC	Asian	12	43 ± 12	5 (42)	12 (100)	0 (0)	-	-	2 (17)
	Healthy	Asian	10	50 ± 5	6 (60)	7 (78)	0 (0)	-	-	-
	Relatives	Asian	8	34 ± 9	3 (38)	6 (75)	8 (100)	-	-	-
		Total	90	42 ± 13	41 (46)	61 (68)	11 (12)	11 (52)	16 (76)	6 (19)

CD: Crohn's disease; UC: Ulcerative colitis; Severe behaviour: Stricturing or penetrating disease.

compared using Fisher's exact or χ^2 test where appropriate. Continuous parameters were assessed as means if normally distributed (compared using one way ANOVA), and medians if not normally distributed (compared using Mann-Whitney *U* test). The software Graphpad Prism 5 and SPSS 21 were used for analyses. $P < 0.05$ was considered statistically significant.

RESULTS

Demographics

Ninety participants (21 CD, 32 UC, 29 healthy controls, and 8 relatives of IBD patients) were divided according to geography, ethnicity and disease (Table 1). All Asian patients were Han Chinese. There was no significant difference when comparing age or gender distribution between countries (Australian *vs* HK subjects), or ethnicities (Asian *vs* Caucasian subjects).

CD vs non-CD (UC, healthy subjects and relatives)

Anti-glycan antibody prevalence and number of antibodies positive: As the anti-glycan antibodies are known to be associated with CD, we compared each CD *vs* non-CD groups in combined Australian and HK cohorts. Three (gASCA, ALCA, AMCA) of the four anti-glycan antibodies were present in a significantly higher proportion of Australian Caucasian CD compared to all non-CD subjects combined [6/9 (67%) *vs* 2/69 (3%), $P < 0.001$; 4/9 (44%) *vs* 4/69 (6%), $P = 0.005$; and 6/9 (67%) *vs* 10/69 (15%), $P = 0.002$, respectively]. In contrast, in the HK Asian CD group only AMCA had a significantly higher proportion of subjects positive compared to all non-CD groups combined [7/12 (58%) *vs* 10/69 (15%), $P = 0.002$] (Table 2).

The proportion of subjects with at least one, and at least two, antibodies positive was significantly higher in the Australian Caucasian CD group than all non-CD groups combined [9/9 (100%) *vs* 17/69 (25%), $P < 0.001$; 6/9 (67%) *vs* 5/69 (7%), $P = 0.001$]. The HK Asian CD group had a significantly higher proportion of subjects with at least one antibody positive compared to all non-CD groups combined, [7/12 (58%) *vs* 17/69 (25%), $P = 0.035$], however, only 2/12 (17%) had at least two antibodies positive. All subjects in the HK Asian CD group that had an antibody positive had AMCA as one of the antibodies.

Anti-glycan antibody titres: The titres of three of the four anti-glycan antibodies (gASCA, ALCA, and AMCA), and the quartile sum score (QSS), were significantly higher in the Australian Caucasian CD group than all non-CD groups combined (median titres 59 *vs* 9, $P < 0.001$; 45 *vs* 18, $P = 0.002$; 111 *vs* 67, $P = 0.002$; 14 *vs* 9, $P < 0.001$, respectively). Two of the four anti-glycan antibodies (ALCA, and AMCA), and the QSS, had significantly higher titres in the HK Asian Crohn's group than all non-CD groups combined (median titres 27 *vs* 18, $P = 0.029$; 121 *vs* 67, $P = 0.003$, 13 *vs* 9, $P = 0.022$, respectively). HK relatives did not have a significantly higher number of antibodies positive, or a higher antibody titre, than other healthy subjects.

CD in Australian Caucasians and Hong Kong Asians

Anti-glycan antibody prevalence and number of antibodies positive: The proportion of subjects with positive gASCA was significantly higher in the Australian Caucasian CD group than the HK Asian CD group [6/9 (67%) *vs* 0/12 (0%), $P < 0.001$]. Prevalence of ALCA, ACCA and AMCA in Australian Caucasian CD patients [4/9 (44%), 2/9 (22%), and 6/9 (67%)] was not significantly different to the HK CD patients [1/12 (8%), 1/12 (8%) and 7/12 (58%)]. The proportion of subjects with at least one antibody, or at least two antibodies, positive was significantly higher in Australian Caucasian CD patients than the HK Asian CD patients [9/9 (100%) *vs* 7/12 (58%), $P = 0.045$; 6/9 (67%) *vs* 2/12 (17%), $P = 0.032$].

Anti-glycan antibody titres: A significant difference was seen when comparing gASCA titres of Australian Caucasian CD to HK Asian CD patients (median titres 59 *vs* 9, $P = 0.002$). There was no significant difference in any other antibody titre, or the QSS, between the CD patients in the two countries.

Table 2 Antibody positivity and titre according to geography, ethnicity and disease *n* (%)

	Australia					Hong Kong (all Asian)			
	CD	UC		Healthy		CD	UC	Healthy	Relatives
	Caucasian	Caucasian	Asian	Caucasian	Asian				
Total	9	10	10	10	9	12	12	10	8
Antibody positivity									
gASCA	6 (67)[a,c]	0 (0)	1 (10)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ALCA	4 (44)[a]	1 (10)	0 (0)	2 (20)	0 (0)	1 (8)	1 (8)	0 (0)	0 (0)
ACCA	2 (22)	1 (10)	0 (0)	2 (20)	3 (33)	1 (8)	1 (8)	1 (10)	0 (0)
AMCA	6 (67)[a]	0 (0)	0 (0)	1 (10)	3 (33)	7 (58)[a]	2 (17)	2 (20)	2 (25)
pANCA	0 (0)	7 (70)[e]	5 (50)[e]	0 (0)	1 (11)	3 (25)	4 (33)[e]	0 (0)	0 (0)
No. of positive antibodies									
At least 1	9 (100)[a,c]	2 (20)	1 (10)	2 (20)	4 (44)	7 (58)[a]	4 (33)	2 (20)	2 (25)
At least 2	6 (67)[a,c]	0 (0)	0 (0)	2 (20)	2 (22)	2 (17)	0 (0)	1 (10)	0 (0)
Antibody/QSS titre median (range)									
gASCA	59 (146)[a,c]	10 (12)	9 (47)	17 (45)	8 (21)	9 (44)	2 (39)	11 (17)	5 (46)
ALCA	46 (79)[a]	17 (86)	14 (38)	23 (77)	18 (34)	27 (69)[a]	17 (82)	17 (18)	24 (25)
ACCA	50 (188)	39 (101)	43 (56)	60 (310)	76 (135)	50 (80)	37 (87)	46 (77)	34 (62)
AMCA	111 (154)[a]	63 (47)	70 (60)	79 (59)	75 (116)	121 (459)[a]	60 (272)	59 (145)	74 (94)
QSS	14 (5)[a]	9 (6)	10 (6)	12 (8)	9 (10)	13 (10)[a]	8 (7)	8 (10)	10 (9)

[a]Significantly higher than all non-Crohn's disease (CD) groups combined ($P < 0.05$); [c]Significantly higher than Hong Kong Asian CD ($P < 0.05$); [e]Significantly higher than all non-ulcerative colitis (UC) groups combined ($P < 0.05$). gASCA: Anti-*Saccharomyces cerevisiae*; ALCA: Anti-laminaribioside carbohydrate IgG antibodies; ACCA: Anti-chitobioside carbohydrate IgA antibodies; AMCA: Anti-mannobioside carbohydrate IgG antibodies; pANCA: Atypical perinuclear anti-neutrophil cytoplasmic antibody; QSS: Quartile sum score.

pANCA presence

The proportion of subjects with a positive pANCA in the Australian Caucasian UC group (7/10, 70%) did not differ significantly from the Australian Asian UC (5/10, 50%) and the HK Asian UC (4/12, 33%) patients. pANCA was present in 3/12 (25%) of the HK Asian CD group, but was virtually absent from all other non-UC groups. When comparing the Australian Caucasian UC patients, Australian Asian UC patients, and the HK Asian UC patients, to all non-UC subjects combined, each UC group had a statistically higher proportion of subjects with a positive pANCA ($P < 0.001$, $P = 0.002$, $P = 0.025$, respectively).

DISCUSSION

There are very few studies reporting the prevalence of antibodies to microbial antigens in non-Western countries and between different ethnicities. This is the first report investigating anti-glycan antibodies in an Asian cohort, and the first report investigating pANCA in an Asian cohort residing in a country outside of Asia.

The prevalence of anti-glycan antibody in Australian Caucasian CD patients was consistent with previous published Western CD cohorts[14], and were more prevalent than in all other subjects studied. The exception was ACCA which had a high prevalence in the healthy Australian Asian (33%) and Caucasian (20%) subjects, in contrast to a previously reported lower prevalence (0.5%-12%) in other healthy cohorts[2].

gASCA was not present in any HK Asian CD subjects studied. This is in contrast to Asian data showing a similar prevalence of ASCA in Japanese[15] and South Korean[16,17] CD patients to that of Caucasian CD cohorts. A low gASCA titre was present in HK subjects. Chinese patients in HK may not raise an antibody response to this antigen, or may do it only in low titre. Lawrence *et al*[18] directly compared a HK IBD cohort with an Australian Caucasian IBD cohort and found ASCA IgG detection was similar but IgA was lower in Chinese CD patients. This IgG detection may differ from the gASCA IgG we measured, although the two antibody measurements have been said to correlate well[6].

Differences in prevalence of the anti-glycan antibodies may reflect true pathogenic differences in different populations. However they may still be present in some populations in low titre; this may need to be taken into account in non-Caucasian ethnicities.

AMCA was prevalent in Asian IBD patients and healthy Asian subjects. This antibody has low specificity for differentiating IBD from health in an Asian population. Bernstein *et al*[19] demonstrated a similar lack of specificity in a Canadian study of Caucasian and First Nations cohorts. He found a relatively high prevalence of IBD associated antibodies (pANCA, ASCA, anti-OmpC, anti-I2, and anti-CBir-1) in all First Nations cohorts (including controls). They concluded that these antibodies are unlikely to be of pathogenic significance.

pANCA was less prevalent in Asian UC than Caucasian UC patients. The lack of significance may relate to the small number of subjects studied and the modest difference observed. These findings are consistent with Asian UC studies from Japan (35%)[20], South Korea (22%)[21], and HK (44%)[18]. The prevalence in our Caucasian UC cohort was consistent with other Western UC cohorts[2].

Our study included 8 first-degree relatives of IBD patients (all Asian from HK), six related to CD patients, and 2 to UC patients. The only 2 relatives with a positive anti-glycan antibody were related to a CD patient, and for both it was a positive AMCA. There have been no studies of

antibodies in relatives of IBD patients in Asian cohorts, however several studies have shown ASCA is present in 20%-56% of Caucasian healthy relatives of patients with CD[22-28]. None of the 8 relatives had a positive pANCA. Early studies of Caucasians demonstrated pANCA presence in 15%-30% of first degree relatives of patients with UC[29,30], however this has not been replicated[31-36], or not been significant when comparing to healthy non-related controls[37].

This study has a number of limitations. Sample sizes were small; however these data provide a basis for larger confirmatory studies. Australian Caucasian CD patients had more severe disease than Hong Kong Asian CD patients which could be contributing to differences in antibodies[38], however, because of the small numbers, comparisons between antibodies and CD phenotype were not made, but should be considered in further studies. Our lack of Australian Asian CD subjects limited our ability to separately determine the effects of ethnicity and geography. A cross sectional study on serological antibodies may be limited by changes in antibody status over time, although it appears that seropositive/seronegative antibody status remains relatively stable over time for the individual antibodies ASCA[13,14,23,38-41], ALCA, ACCA and AMCA[14,38,42].

In conclusion serological antibodies associated with IBD appear to differ in their presence and titre between the West and Chinese IBD patients. Caution should therefore be exercised in attributing pathogenic importance or using them as prognostic markers in different ethnic and geographic patient populations[43-45].

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COMMENTS

Background

Serological antibodies to enteric antigens are a hallmark of inflammatory bowel disease (IBD) and may carry pathogenic and prognostic significance.

Research frontiers

Until two decades ago IBD was rare in Asia, but recent population-based and referral centre cohorts have shown a rising incidence and prevalence of IBD in Asia.

Innovations and breakthroughs

Although there has been previous research on serological antibodies in Caucasian patients with IBD, there is limited information about their role and prevalence in Asian patients in Asia, or in Asian migrants to the West.

Applications

This study has found that serological antibodies associated with IBD appear to differ in their presence and titre between Western and Chinese IBD patients. Caution should therefore be exercised in attributing pathogenic importance or using them as prognostic markers in different ethnic and geographic patient populations.

Terminology

Anti-*Saccharomyces cervisiae* antibodies, which are directed against the cell wall mannan of the yeast *Saccharomyces*, that shares homology with intestinal bacteria; Antiglycan antibodies, which are directed against carbohydrates found on immune cells, erythrocytes, tissue matrices and microorganisms, and likely reflect the interaction between the immune system and glycosylated cell wall components of microbiota. The anti-glycan antibodies include: anti-chitobioside, anti-laminaribioside and anti-mannobioside; Perinuclear anti-neutrophil cytoplasmic antibody is widely regarded as a marker of ulcerative colitis.

Peer review

This is interesting data of a little studied area in inflammatory bowel disease. The subject matter may be a spring board to further studies and understanding of the pathogenesis and prognosis of IBD.

REFERENCES

1 **Danese S**, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. *World J Gastroenterol* 2006; **12**: 4807-4812 [PMID: 16937461]
2 **Prideaux L**, De Cruz P, Ng SC, Kamm MA. Serological antibodies in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2012; **18**: 1340-1355 [PMID: 22069240 DOI: 10.1002/ibd.21903]
3 **Dotan N**, Altstock RT, Schwarz M, Dukler A. Anti-glycan antibodies as biomarkers for diagnosis and prognosis. *Lupus* 2006; **15**: 442-450 [PMID: 16898180 DOI: 10.1191/0961203306lu2331oa]
4 **Sendid B**, Colombel JF, Jacquinot PM, Faille C, Fruit J, Cortot A, Lucidarme D, Camus D, Poulain D. Specific antibody response to oligomannosidic epitopes in Crohn's disease. *Clin Diagn Lab Immunol* 1996; **3**: 219-226 [PMID: 8991640]
5 **Dotan I**, Fishman S, Dgani Y, Schwartz M, Karban A, Lerner A, Weishauss O, Spector L, Shtevi A, Altstock RT, Dotan N, Halpern Z. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006; **131**: 366-378 [PMID: 16890590 DOI: 10.1053/j.gastro.2006.04.030]
6 **Papp M**, Altorjay I, Dotan N, Palatka K, Foldi I, Tumpek J, Sipka S, Udvardy M, Dinya T, Lakatos L, Kovacs A, Molnar T, Tulassay Z, Miheller P, Norman GL, Szamosi T, Papp J, Lakatos PL. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008; **103**: 665-681 [PMID: 18047543 DOI: 10.1111/j.1572-0241.2007.01652.x]
7 **Schwarz M**, Spector L, Gargir A, Shtevi A, Gortler M, Altstock RT, Dukler AA, Dotan N. A new kind of carbohydrate array, its use for profiling antiglycan antibodies, and the discovery of a novel human cellulose-binding antibody. *Glycobiology* 2003; **13**: 749-754 [PMID: 12851287 DOI: 10.1093/glycob/cwg091]
8 **Quinton JF**, Sendid B, Reumaux D, Duthilleul P, Cortot A, Grandbastien B, Charrier G, Targan SR, Colombel JF, Poulain D. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1998; **42**: 788-791 [PMID: 9691915 DOI: 10.1136/gut.42.6.788]
9 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517 [PMID: 15168363 DOI: 10.1053/j.gastro.2004.01.063]
10 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID:

22001864]
11 **Ng SC**, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, Li MF, Ng KK, Kamm MA, Studd C, Bell S, Leong R, de Silva HJ, Kasturiratne A, Mufeena MN, Ling KL, Ooi CJ, Tan PS, Ong D, Goh KL, Hilmi I, Pisespongsa P, Manatsathit S, Rerknimitr R, Aniwan S, Wang YF, Ouyang Q, Zeng Z, Zhu Z, Chen MH, Hu PJ, Wu K, Wang X, Simadibrata M, Abdullah M, Wu JC, Sung JJ, Chan FK. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* 2013; **145**: 158-165.e2 [PMID: 23583432]
12 **Prideaux L**, Kamm MA, De Cruz PP, Chan FK, Ng SC. Inflammatory bowel disease in Asia: a systematic review. *J Gastroenterol Hepatol* 2012; **27**: 1266-1280 [PMID: 22497584 DOI: 10.1111/j.1440-1746.2012.07150.x]
13 **Landers CJ**, Cohavy O, Misra R, Yang H, Lin YC, Braun J, Targan SR. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002; **123**: 689-699 [PMID: 12198693 DOI: 10.1053/gast.2002.35379]
14 **Rieder F**, Schleder S, Wolf A, Dirmeier A, Strauch U, Obermeier F, Lopez R, Spector L, Fire E, Yarden J, Rogler G, Dotan N, Klebl F. Association of the novel serologic anti-glycan antibodies anti-laminarin and anti-chitin with complicated Crohn's disease behavior. *Inflamm Bowel Dis* 2010; **16**: 263-274 [PMID: 19653286]
15 **Hisabe T**, Matsui T, Sakurai T, Murakami Y, Tanabe H, Matake H, Yao T, Kamachi S, Iwashita A. Anti-Saccharomyces cerevisiae antibodies in Japanese patients with inflammatory bowel disease: diagnostic accuracy and clinical value. *J Gastroenterol* 2003; **38**: 121-126 [PMID: 12640524 DOI: 10.1007/s005350300020]
16 **Kim BG**, Kim YS, Kim JS, Jung HC, Song IS. Diagnostic role of anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic antibodies in patients with inflammatory bowel disease. *Dis Colon Rectum* 2002; **45**: 1062-1069 [PMID: 12195191 DOI: 10.1007/s10350-004-6361-3]
17 **Kim BC**, Park S, Han J, Kim JH, Kim TI, Kim WH. Clinical significance of anti-Saccharomyces cerevisiae antibody (ASCA) in Korean patients with Crohn's disease and its relationship to the disease clinical course. *Dig Liver Dis* 2007; **39**: 610-616 [PMID: 17531556 DOI: 10.1016/j.dld.2007.03.006]
18 **Lawrance IC**, Murray K, Hall A, Sung JJ, Leong R. A prospective comparative study of ASCA and pANCA in Chinese and Caucasian IBD patients. *Am J Gastroenterol* 2004; **99**: 2186-2194 [PMID: 15555001 DOI: 10.1111/j.1572-0241.2004.40486.x]
19 **Bernstein CN**, El-Gabalawy H, Sargent M, Landers C, Rawsthorne P, Elias B, Targan SR. Assessing inflammatory bowel disease-associated antibodies in Caucasian and First Nations cohorts. *Can J Gastroenterol* 2011; **25**: 269-273 [PMID: 21647462]
20 **Kaneko K**, Suzuki Y, Shimizu T, Yamashiro Y, Yabuta K, Lifschitz CH. Anti-neutrophil cytoplasmic antibodies in Japanese children with ulcerative colitis. *J Paediatr Child Health* 1995; **31**: 336-338 [PMID: 7576894 DOI: 10.1111/j.1440-1754.1995.tb00823.x]
21 **Lee JH**, Cheon JH, Kim ES, Chung MJ, Kang W, Kim DH, Ha YJ, Park JJ, Kim TI, Kim WH. The prevalence and clinical significance of perinuclear anti-neutrophil cytoplasmic antibody in Korean patients with ulcerative colitis. *Dig Dis Sci* 2010; **55**: 1406-1412 [PMID: 19507028]
22 **Sendid B**, Quinton JF, Charrier G, Goulet O, Cortot A, Grandbastien B, Poulain D, Colombel JF. Anti-Saccharomyces cerevisiae mannan antibodies in familial Crohn's disease. *Am J Gastroenterol* 1998; **93**: 1306-1310 [PMID: 9707056 DOI: 10.1111/j.1572-0241.1998.00415.x]
23 **Vermeire S**, Peeters M, Vlietinck R, Joossens S, Den Hond E, Bulteel V, Bossuyt X, Geypens B, Rutgeerts P. Anti-Saccharomyces cerevisiae antibodies (ASCA), phenotypes of IBD, and intestinal permeability: a study in IBD families. *Inflamm Bowel Dis* 2001; **7**: 8-15 [PMID: 11233666 DOI: 10.1097/00054725-200102000-00002]
24 **Seibold F**, Stich O, Hufnagl R, Kamil S, Scheurlen M. Anti-Saccharomyces cerevisiae antibodies in inflammatory bowel disease: a family study. *Scand J Gastroenterol* 2001; **36**: 196-201 [PMID: 11252413 DOI: 10.1080/00365520120969]
25 **Annese V**, Andreoli A, Andriulli A, Dinca R, Gionchetti P, Latiano A, Lombardi G, Piepoli A, Poulain D, Sendid B, Colombel JF. Familial expression of anti-Saccharomyces cerevisiae Mannan antibodies in Crohn's disease and ulcerative colitis: a GISC study. *Am J Gastroenterol* 2001; **96**: 2407-2412 [PMID: 11513182 DOI: 10.1111/j.1572-0241.2001.04043.x]
26 **Glas J**, Török HP, Vilsmaier F, Herbinger KH, Hoelscher M, Folwaczny C. Anti-saccharomyces cerevisiae antibodies in patients with inflammatory bowel disease and their first-degree relatives: potential clinical value. *Digestion* 2002; **66**: 173-177 [PMID: 12481163 DOI: 10.1159/000066760]
27 **Sutton CL**, Yang H, Li Z, Rotter JI, Targan SR, Braun J. Familial expression of anti-Saccharomyces cerevisiae mannan antibodies in affected and unaffected relatives of patients with Crohn's disease. *Gut* 2000; **46**: 58-63 [PMID: 10601056 DOI: 10.1136/gut.46.1.58]
28 **Hadrich I**, Vandewalle P, Cheikhrouhou F, Makni F, Krichen MS, Sendid B, Standaert-Vitse A, Ayadi A, Poulain D. Ethnic and socio-cultural specificities in Tunisia have no impact on the prevalence of anti-Saccharomyces cerevisiae antibodies in Crohn's disease patients, their relatives or associated clinical factors. *Scand J Gastroenterol* 2007; **42**: 717-725 [PMID: 17505994 DOI: 10.1080/00365520601083625]
29 **Seibold F**, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology* 1994; **107**: 532-536 [PMID: 8039629]
30 **Shanahan F**, Duerr RH, Rotter JI, Yang H, Sutherland LR, McElree C, Landers CJ, Targan SR. Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. *Gastroenterology* 1992; **103**: 456-461 [PMID: 1634063]
31 **Lee JC**, Lennard-Jones JE, Cambridge G. Antineutrophil antibodies in familial inflammatory bowel disease. *Gastroenterology* 1995; **108**: 428-433 [PMID: 7835584 DOI: 10.1016/0016-5085(95)90070-5]
32 **Folwaczny C**, Noehl N, Endres SP, Loeschke K, Fricke H. Antineutrophil and pancreatic autoantibodies in first-degree relatives of patients with inflammatory bowel disease. *Scand J Gastroenterol* 1998; **33**: 523-528 [PMID: 9648993 DOI: 10.1080/00365529850172106]
33 **Bansi DS**, Lo S, Chapman RW, Fleming KA. Absence of antineutrophil cytoplasmic antibodies in relatives of UK patients with primary sclerosing cholangitis and ulcerative colitis. *Eur J Gastroenterol Hepatol* 1996; **8**: 111-116 [PMID: 8723413 DOI: 10.1097/00042737-199602000-00004]
34 **Reumaux D**, Delecourt L, Colombel JF, Noël LH, Duthilleul P, Cortot A. Anti-neutrophil cytoplasmic autoantibodies in relatives of patients with ulcerative colitis. *Gastroenterology* 1992; **103**: 1706 [PMID: 1426894]
35 **Papo M**, Quer JC, Pastor RM, García-Pardo G, Prats E, Mirapeix E, Rodríguez R, Richart C. Antineutrophil cytoplasmic antibodies in relatives of patients with inflammatory bowel disease. *Am J Gastroenterol* 1996; **91**: 1512-1515 [PMID: 8759652]
36 **Osangthamnont C**, Manatsathit S, Pongprasopchai S, Viriyataveekul R, Chaihirunkarn S, Leelakusolvong S, Boonyapisit S. Antibodies to neutrophil cytoplasma in patients with ulcerative colitis and their first-degree relatives in Thailand. *J Gastroenterol Hepatol* 2001; **16**: 866-871 [PMID: 11555099 DOI: 10.1046/j.1440-1746.2001.02546.x]
37 **Yang P**, Järnerot G, Danielsson D, Tysk C, Lindberg E. P-ANCA in monozygotic twins with inflammatory bowel

disease. *Gut* 1995; **36**: 887-890 [PMID: 7615278 DOI: 10.1136/gut.36.6.887]
38 **Rieder F**, Schleder S, Wolf A, Dirmeier A, Strauch U, Obermeier F, Lopez R, Spector L, Fire E, Yarden J, Rogler G, Dotan N, Klebl F. Serum anti-glycan antibodies predict complicated Crohn's disease behavior: a cohort study. *Inflamm Bowel Dis* 2010; **16**: 1367-1375 [PMID: 20024902 DOI: 10.1002/ibd.21179]
39 **Desir B**, Amre DK, Lu SE, Ohman-Strickland P, Dubinsky M, Fisher R, Seidman EG. Utility of serum antibodies in determining clinical course in pediatric Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 139-146 [PMID: 15017619 DOI: 10.1016/S1542-3565(03)00321-5]
40 **Israeli E**, Grotto I, Gilburd B, Balicer RD, Goldin E, Wiik A, Shoenfeld Y. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut* 2005; **54**: 1232-1236 [PMID: 16099791 DOI: 10.1136/gut.2004.060228]
41 **Müller S**, Styner M, Seibold-Schmid B, Flogerzi B, Mähler M, Konrad A, Seibold F. Anti-Saccharomyces cerevisiae antibody titers are stable over time in Crohn's patients and are not inducible in murine models of colitis. *World J Gastroenterol* 2005; **11**: 6988-6994 [PMID: 16437604]
42 **Rieder F**, Lopez R, Franke A, Wolf A, Schleder S, Dirmeier A, Schirbel A, Rosenstiel P, Dotan N, Schreiber S, Rogler G, Klebl F. Characterization of changes in serum anti-glycan antibodies in Crohn's disease--a longitudinal analysis. *PLoS One* 2011; **6**: e18172 [PMID: 21573154 DOI: 10.1371/journal.pone.0018172]
43 **Devlin SM**, Yang H, Ippoliti A, Taylor KD, Landers CJ, Su X, Abreu MT, Papadakis KA, Vasiliauskas EA, Melmed GY, Fleshner PR, Mei L, Rotter JI, Targan SR. NOD2 variants and antibody response to microbial antigens in Crohn's disease patients and their unaffected relatives. *Gastroenterology* 2007; **132**: 576-586 [PMID: 17258734 DOI: 10.1053/j.gastro.2006.11.013]
44 **Cruyssen BV**, Peeters H, Hoffman IE, Laukens D, Coucke P, Marichal D, Cuvelier C, Remaut E, Veys EM, Mielants H, De Vos M, De Keyser F. CARD15 polymorphisms are associated with anti-Saccharomyces cerevisiae antibodies in caucasian Crohn's disease patients. *Clin Exp Immunol* 2005; **140**: 354-359 [PMID: 15807862 DOI: 10.1111/j.1365-2249.2005.02759.x]
45 **Mow WS**, Vasiliauskas EA, Lin YC, Fleshner PR, Papadakis KA, Taylor KD, Landers CJ, Abreu-Martin MT, Rotter JI, Yang H, Targan SR. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004; **126**: 414-424 [PMID: 14762777 DOI: 10.1053/j.gastro.2003.11.015]

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BRIEF ARTICLE

Incidence and characteristics of HBV reactivation in hematological malignant patients in south Egypt

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Abstract

AIM: To investigate characteristics of hepatitis B virus (HBV) implicated in HBV reactivation in patients with hematological malignancies receiving immunosuppressive therapy.

METHODS: Serum samples were collected from 53 patients with hematological malignancies negative for hepatitis B surface antigen (HBsAg) before the start of and throughout the chemotherapy course. HBV reactivation was diagnosed when the HBsAg status changed from negative to positive after the initiation of chemotherapy and/or when HBV DNA was detected by real-time detection polymerase chain reaction (RTD-PCR). For detecting the serological markers of HBV infection, HBsAg as well as antibodies to the core antigen (anti-HBc) and to the surface antigen were measured in the sera by CEIA. Nucleic acids were extracted from sera, and HBV DNA sequences spanning the S gene were amplified by RTD-PCR. The extracted DNA was further subjected to PCR to amplify the complete genome as well as the specific genomic sequences bearing the enhancer Ⅱ/core promoter/pre-core/core regions (nt 1628-2364). Amplicons were sequenced directly.

RESULTS: Thirty-five (66%) of the 53 HBsAg-negative patients were found to be negative serologically for anti-HBc, and the remaining 18 (34%) patients were positive for anti-HBc. Five of the 53 (9.4%) patients with hematologic malignancies experienced HBV reactivation. Genotype D1 was detected in all five patients. Four types of mutant strains were detected in the S gene product of HBV strains and were isolated from 3 patients with HBV reactivation: T/S120, L143, and I126. HBV DNA was detected in the pretreatment HBsAg-negative samples in one of the five patients with HBV reactivation. In this patient, sequences encompassing the HBV full genome obtained from sera before the start of chemotherapy and at the time of *de novo* HBV hepatitis were detected and it showed 100% homology. Furthermore, in the phylogenetic tree, the sequences were clustered together, thereby indicating that this patient developed reactivation from an occult HBV infection.

CONCLUSION: Past infection with HBV is a risk factor for HBV reactivation in Egypt. Mandatory anti-HBc

screening prior to chemotherapy in patients with hematological malignancies is recommended.

Key words: Hepatitis B virus; Occult infection; Reactivation; Hepatitis B surface antigen

Core tip: The study aimed to investigate characteristics of hepatitis B virus (HBV) implicated in HBV reactivation in patients with hematological malignancies receiving immunosuppressive therapy in Egypt. Fifty-three hepatitis B surface antigen (HBsAg)-negative patients treated with chemotherapy were included in the study. The incidence of HBV reactivation was 9.4% among the studied cohort, and all of the affected individuals were positive for HBsAg as well as antibodies to the hepatitis B core antigen. The present study provides further evidence via molecular evolutionary analysis of the development of HBV reactivation from an occult HBV infection. Past infection with HBV is a risk factor for HBV reactivation in Egypt. Mandatory antibodies to the core antigen screening prior to chemotherapy in patients with hematological malignancies is suggested.

Elkady A, Aboulfotuh S, Ali EM, Sayed D, Abdel-Aziz NM, Ali AM, Murakami S, Iijima S, Tanaka Y. Incidence and characteristics of HBV reactivation in hematological malignant patients in south Egypt. *World J Gastroenterol* 2013; 19(37): 6214-6220 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6214.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6214

INTRODUCTION

Infection with hepatitis B remains one of the major causes of acute and chronic liver disease. An estimated 350-400 million people are chronically infected with hepatitis B virus (HBV) worldwide[1].

The reactivation of hepatitis B infection has been recorded in many clinical settings: chronic HBV infection after the cessation of HBV treatment, patients with malignant disease who receive immunosuppressant or chemotherapy, patients with end stage renal failure, and patients co-infected with human immunodeficiency virus (HIV)[2-6]. Patients with resolved HBV infection are diagnosed serologically by clearance of serum hepatitis B surface antigen (HBsAg) and the appearance of the hepatitis B core antibody (anti-HBc), with or without antibodies to hepatitis B surface antigen (anti-HBs)[7]. These patients are at risk of hepatitis B reactivation due to any factor that can suppress the immune system[8,9]. *De novo* hepatitis B is of particular concern in this subset of patients because it commonly leads to severe liver dysfunction and fatal hepatitis[10,11].

Occult hepatitis B is defined by the presence of HBV DNA in the serum or the liver in the absence of HBsAg, with or without anti-HBc or anti-HBs. In these patients, a low level of HBV replication has been shown to persist in the liver and in peripheral blood mononuclear cells for decades[12]. Occult HBV infection is observed worldwide, and its prevalence is related closely to the endemicity of HBV infection.

Large scale geographic heterogeneity in the prevalence of HBV had been reported worldwide. Africa is one of the highly endemic regions of HBV, and an intermediate endemicity of HBV infection had been recorded in Egypt[13,14].

The aim of this study was to investigate the incidence of HBV reactivation and the underlying risk factors of hepatitis B reactivation in Egyptian patients who received cytotoxic chemotherapy for hematological malignancies.

MATERIALS AND METHODS

Patients

Fifty-nine consecutive patients with hematological malignancies were admitted to the oncology department of Sohag Faculty of Medicine and South Egypt Cancer Institution from November 2010 to October 2011. After admission, all patients underwent physical examination and blood and serum biochemistry analyses. All of patients received chest computed tomography and ultrasonography of the abdomen as an initial evaluation.

In clinical practice, patients are monitored during chemotherapy using liver function tests. HBsAg and HBV DNA are tested in patients with elevated liver enzymes. For the purpose of this study, serum samples were collected before and after the start of the chemotherapy course. The collected sera were stored at -80 ℃ for future examination of HBsAg, anti-HBs, and anti-HBc. HBV reactivation was diagnosed when the HBsAg status changed from negative to positive after the initiation of chemotherapy and/or when HBV DNA was detected as measured by real-time detection polymerase chain reaction (RTD-PCR) using stored samples from patients, as described latter.

Serological markers of HBV infection

HBsAg was measured by enzyme immunoassay (EIA) (AxSYM; Abbott Japan, Tokyo, Japan) or chemiluminescence enzyme immunoassay (CLEIA) (Fujirebio, Tokyo; Japan). Anti-HBc of the IgG class was determined by radioimmunoassay (Abbott Japan). All serologic assays were performed according to the manufacturer's instructions.

Detection and quantitation of serum HBV DNA

HBV-DNA sequences spanning the S gene were amplified by RTD-PCR according to the previously described protocol with a slight modification and a detection limit of 100 copies/mL (equivalent to 20 IU/mL)[15].

Sequencing and molecular evolutionary analysis of HBV

Nucleic acids were extracted from serum samples (200 μL) using the QIAamp DNA extraction kit (Qiagen, Hilden,

Table 1 Characteristics of 53 patients with malignant hematologic disease who were negative for hepatitis B surface antigen *n* (%)

Characteristics	Total (*n* = 53)	Anti-HBc positive (*n* = 18)	Anti-HBc negative (*n* = 35)	*P* value
Age yr, mean ± SD	27.8 ± 26.2	34.4 ± 27.9	27.7 ± 25.4	0.42
Gender (male)	26 (49.1)	10 (55.6)	16 (45.7)	0.56
Diagnosis				
Malignant lymphoma	26 (40.1)	9 (50.0)	17 (48.6)	1.00
Acute leukemia	25 (47.2)	9 (50.0)	15 (42.9)	0.77
Chronic leukemia	1 (1.9)	0 (0.0)	1 (2.9)	1.00
Multiple myeloma	1 (1.9)	0 (0.0)	1 (2.9)	1.00

Anti-HBc: Antibody to hepatitis B core antigen.

Germany).

Extracted DNA was subjected to PCR for amplifying the complete genome and the specific genomic sequences bearing enhancer Ⅱ/core promoter/pre-core/core regions (nt 1628-2364), as described previously[16].

Amplicons were sequenced directly using the ABI Prism Big Dye ver. 3.1 kit in the AMI 3100 DNA automated sequencer (Applied Biosystems; Foster City, CA, United States).

All sequences were analyzed in both the forward and reverse directions. HBV genotypes were determined by molecular evolutionary analysis. Reference HBV sequences were retrieved from the DDBJ/EMBL/GenBank database and aligned by CLUSTALX, and genetic distances were estimated with the 6-parameter method in the Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp/)[17]. Based on the obtained distances, phylogenetic trees were constructed by the neighbor-joining (NJ) method with the mid-point rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1000 times for analysis by the ODEN program of the National Institute of Genetics.

Ethical consideration

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and its subsequent amendments, and informed consent was obtained from all patients.

Statistical analysis

Statistical analysis was performed with the Fisher's exact probability test and the independent *t* test for the continuous variables using the SPSS software package (SPSS, Chicago, IL, United States). *P* values (two-tailed) less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Six of the 59 patients with hematologic malignancies were found to be HBsAg positive and were excluded from the analysis. Therefore, a total of 53 HBsAg-negative patients were checked for the serological markers of infection with hepatitis B. The background general characteristics of the 53 HBsAg-negative patients are presented in Table 1. The mean age of the analyzed cohort was 27.8 ± 26.2 years old. Thirty-five (66%) of 53 HBsAg-negative patients were found to be anti-HBc-negative, and 18 (34%) patients were serologically positive for anti-HBc. The predominance of male patients was observed in both the anti-HBc-positive and -negative patient groups. Twenty-six patients (40.1%) were diagnosed with malignant lymphoma, whereas 25 patients (47.2%) were diagnosed with acute leukemia. Solitary cases of chronic leukemia and multiple myeloma were also included in the studied cohort. An insignificantly higher incidence of acute leukemia cases was observed in the anti-HBc-positive patients (9/18; 50%) compared with the anti-HBc-negative patients (15/35; 42.9%).

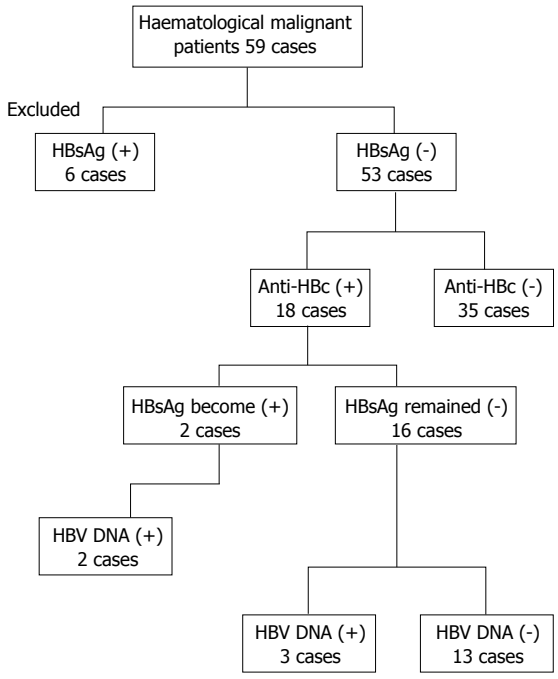

Figure 1 Longitudinal representation of hepatitis B reactivation after chemotherapy in patients with hematological malignancies. HBsAg: Hepatitis B surface antigen; anti-HBc: Antibody to hepatitis B core antigen; HBV: Hepatitis B virus.

Consequences of HBV serology after receiving anti-cancer treatment

After the initiation of systemic chemotherapy, examination of the HBV serology revealed that two (3.8%) of the HBsAg-negative patients became serologically positive for HBsAg. In addition, 3 more patients (5.8%) exhibited detectable HBV DNA in their sera after the start of the anticancer therapy (Figure 1). Interestingly, none of the serologically negative patients for anti-HBc became serologically positive for HBsAg or molecularly detectable for HBV DNA. In contrast, 2 of the 18 anti-HBc-positive patients (11.1%) became serologically positive for the HBsAg, and 3 (16.7%) became molecularly detectable for the HBV DNA. In brief, 5 of the 53 HBsAg negative

Table 2 Clinical and virological characteristics of patients who experienced hepatitis B reactivation

Characteristics	Case 1	Case 2	Case 3	Case 4	Case 5
Age (yr)/gender	79/F	8/M	11/F	5/M	20/M
Diagnosis	NHL (stage Ⅲ)	AML	ALL	ALL	ALL
Treatment[1]	CVP	St Jude protocol	St Jude protocol	St Jude protocol	St Jude protocol
HBV serology and DNA prior to chemotherapy					
HBsAg/anti-HBs/HBV DNA (log copy/mL)	(-)/(+)/1.8	(-)/(+)/negative	(-)/(-)/negative	(-)/(+)/negative	(-)/(nt)/negative
HBV reactivation months after anti-cancer therapy	12	4	5	6	4
HBV serology and DNA after chemotherapy					
HBsAg/anti-HBs/HBV DNA (log copy/mL)	(+)/(nt)/7.6	(+)/(+)/5.8	(-)/(-)/3.1	(-)/(+)/2.9	(-)/(nt)/2.0
ALT (IU/mL)	35	195	27	86	17
Total bilirubin (mg/dL)	1	1.1	1.3	1.1	0.2
Outcome	Died	Died	Died	Alive	Alive
HBV genotype	D1	D1	D1	D1	D1
Core promoter mutation	Wild	T1764/G1766	A1764	Wild	-
Pre-core A1896	Mutant	Wild	Wild	Wild	-
Amino acid mutation in S gene product	P120S/S143L	P120T	-	T126I	-

M: Male; F: Female; NHL: Non-Hodgkin lymphoma; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; HBsAg: Hepatitis B surface antigen; Anti-HBs: Antibody to hepatitis B surface antigen; CVP: Cyclophosphamide, vincristine, prednisone; ALT: Alanine amino transferase enzyme. [1]St Jude protocol: (1) prephase: vincristine + steroid; (2) induction: vincristine + farmarabin + aracytine + etoposide, intrathecal; (3) consolidation: high dose methotrexate + mercaptopurine; (4) continuation: methotrexate + mercaptopurine.

patients (9.4%), representing 27.8% (5/18) of the anti-HBc-positive patients in the studied cohort, manifested the criteria of HBV reactivation (Figure 1).

Clinical and virological criteria of the patients who manifested HBV reactivation

Five of the 53 patients (9.4%) treated for hematologic malignancies manifested HBV reactivation throughout the anti-cancer therapy regimen. The demographic, clinical and virological criteria of the HBV infection of the five patients who experienced HBV reactivation are summarized in Table 2 (cases 1-5). The mean age of the five patients was 24.6 ± 30.9 years old. Three of the patients were males (cases 2, 4 and 5), and two were females. Four patients were diagnosed with acute leukemia (cases 2-5), and only one patient (case 1) was diagnosed with malignant lymphoma. All of the 5 patients received a steroid regimen as a part of their anticancer therapy. All 5 patients were positive for anti-HBc. Three patients were positive for anti-HBs (cases 1, 2 and 4), and only one patient was serologically negative for the anti-HBs (case 3). Because of small volume of serum sample obtained from case 5, anti-HBs could not be tested. After HBV reactivation, two cases (cases 2 and 4) exhibited abnormal ALT levels, and one patient (case 2) experienced a more than 3-fold increase in the ALT level, indicating the emergence of hepatitis in this patient. None of the 5 cases who experienced had the HBV reactivation after cancer chemotherapy received an antiviral treatment for HBV.

The virological and molecular criteria are summarized in Table 2. The infecting genotype of the HBV strains was HBV genotype D, subtype D1 in all five cases. Two core promoter HBV variants were detected in 2 patients. The two variants were T1764/G1766 and A1764 in cases 2 and 3, respectively. The stop codon pre-core HBV mutant (A1896) was detected in one patient (case 1).

Infection with HBV mutant strains in the S gene product was detected in 3 patients. The amino acid escape mutant strains are as follows: S120 and L143 (case 1), T120 (case 2) and I126 (case 4). Four types of mutant strains (T/S120, L143, and I126) were detected in the S gene strains of 3 patients (cases 1, 2 and 4, respectively).

DNA sequencing and phylogenetic analysis

HBV DNA was quantified retrospectively by RTD-PCR in the stored samples of the five patients with HBV reactivation. Evidence of occult HBV infection at the time of the HBsAg-negative status (before the start of anti-cancer therapy) was detected by RTD-PCR in one patient (case 1). To determine the source of HBV infection, sera from case 1 before (case 1-A) and at the time of HBV reactivation (case 1-B) were subjected to HBV full genome amplification and sequencing. Sequences encompassing the HBV full genome obtained from sera before the start of chemotherapy and at the time of *de novo* HBV hepatitis revealed 100% homology, and the two sequences clustered together in the phylogenetic tree (Figure 2). These results demonstrate that case 1 developed reactivation from an occult HBV infection.

DISCUSSION

This study is considered the first step in documenting and characterizing the reactivation of hepatitis B in Egypt among patients negative for the HBsAg who received immunosuppressive therapy. The current study presented

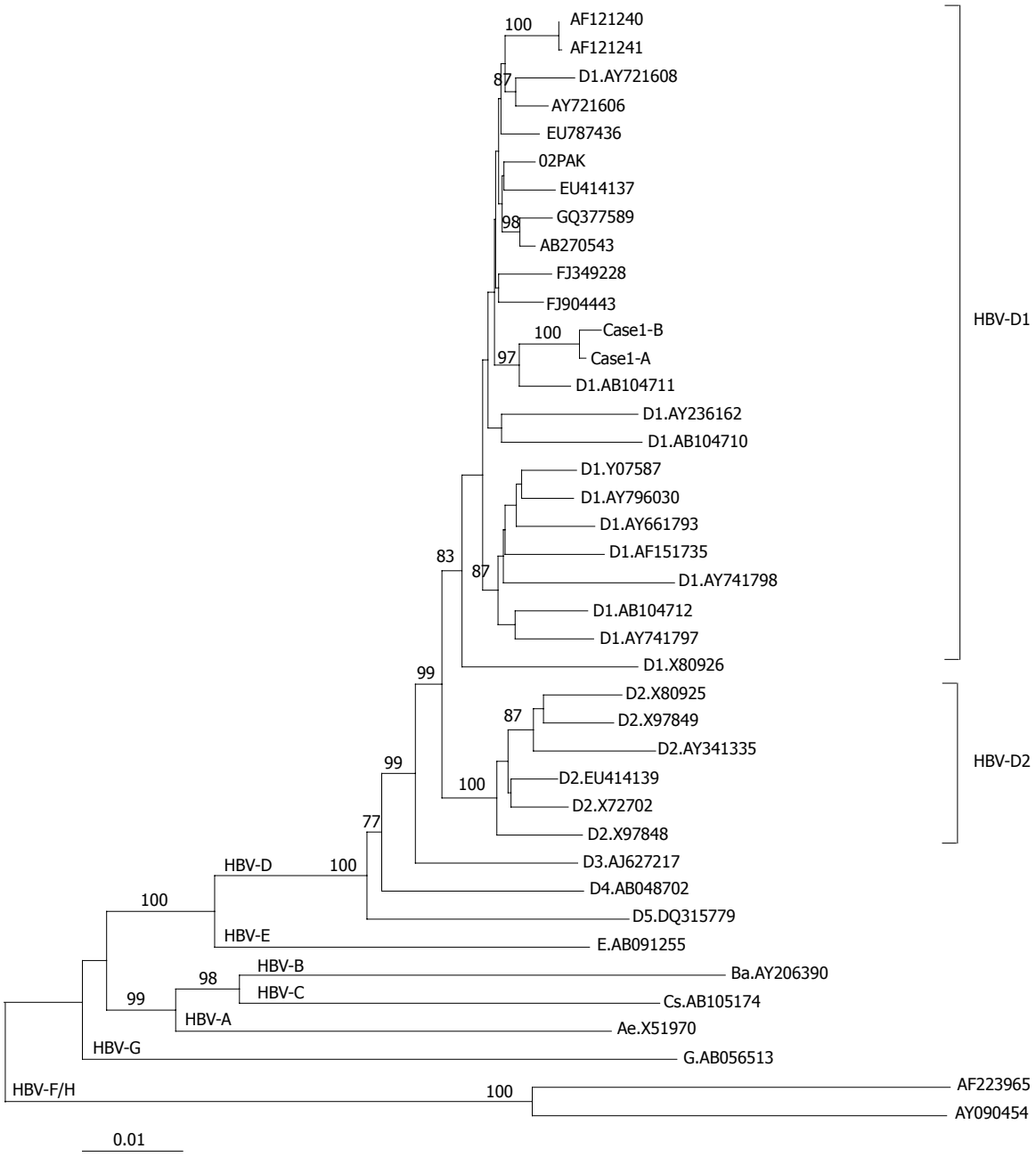

Figure 2 The complete genome of the hepatitis B virus was isolated and sequenced (case 1) prior to the start of chemotherapy (case 1-A) and after the emergence of hepatitis B virus reactivation (case 1-B). The phylogenetic analysis demonstrated that the patient (case 1) developed an hepatitis B virus (HBV) reactivation of an occult HBV infection.

further evidence that resolved hepatitis B infection and occult HBV infection may represent a hidden risk factor for the development of *de novo* hepatitis B.

The incidence of hepatitis B reactivation in the HBsAg-negative group was 9.4%, and all cases of reactivation occurred in patients with resolved or past infection with hepatitis B, as evidenced by the absence of HBsAg and the serological detection of anti-HBc. The patients who had HBV reactivation represent 27% of the HBsAg-negative/anti-HBc-positive patients. This incidence was comparable to the incidence that was described by Hui *et al*[18]. In their study, Hui *et al*[18] described an HBV reactivation incidence of 3.3% (8/244) in their studied cohort, which included HBsAg-negative lymphoma patients receiving systemic chemotherapy. Of note, all 8 patients were seropositive for either anti-HBc or anti-HBs antibody. Recently, Matsue *et al*[19] conducted a retrospective study on consecutive patients with CD20-positive B cell lymphoma before and after rituximab-containing treatment. In the latter study, 5 out of 230 patients negative for HBsAg (2.2%) experienced HBV reactivation, representing an incidence of 8.9% of the anti-HBc-positive patients[19]. In a prospective observational study of patients with hematological malignancies (a study cohort similar to the current study), Francisci *et al*[20] reported the incidence of HBV reactivation was (18%), which is close

to that detected in the present study. The reasons for the difference in the incidence in HBV reactivation among different studies remain to be elucidated. However, the intensity of treatment, patient characteristics, and geographic differences in HBV prevalence and its genotypes may account for these differences[21]. Furthermore, the lack of a clear definition of HBV reactivation should not be ignored as a possible explanation for this variation in the incidence. In this study, the inclusion of patients who had detectable HBV DNA after cancer chemotherapy plus patients who exhibited HBsAg seroconversion after receiving the anticancer therapy dramatically increased the incidence of HBV reactivation among the studied cohort. This criterion of including cases with detectable HBV DNA after cancer chemotherapy as a sign of HBV reactivation was not used to define cases with HBV reactivation in the related studies[18,19]. The variations in the cohort size among the different studies cannot be ignored as a possible factor that may be implicated in such discrepancy.

Occult HBV infection is defined by the detection of HBV DNA in the sera or in the livers of serologically HBsAg-negative patients[14]. Until recently, the clinical effects of occult HBV infection were unclear regarding the influence on the progression of liver disease, the development of hepatocellular carcinoma, the risk for HBV reactivation, and the transmission of HBV infection[22]. The underlying mechanisms for the pathogenesis of occult HBV infection may be due to either viral or host factors[23]. One of the important viral factors is the presence of mutations in the HBV DNA sequence, which may interfere with the detection of HBsAg by the commercial assays, *i.e.*, "escape mutations"[24]. In the present study, 4 types of possible escape mutants were detected in 3 of the 5 patients who experienced HBV reactivation[25]. Previous *in vitro* studies have reported that escape mutations are associated with an increased immune evasive capacity and are capable of causing symptomatic flare up and high viral loads[26]. Furthermore, studying the viral genome isolated from case 1 revealed a complete match of the sequences obtained before the start of chemotherapy and at the time of reactivation. The present study provides further evidence of the emergence of HBV reactivation of occult hepatitis B as confirmed by the molecular evolutionary analysis[27]. Furthermore, two amino acid escape mutations in the S gene product, P120S and S143L, were detected in the HBV viral genome isolated from case 1.

Patients with malignancies in Egypt are monitored only by testing ALT levels throughout the chemotherapy course. Therefore, the present study, which is the first to explore HBV reactivation in Egypt, suggests mandatory serological screening for anti-HBc and anti-HBs in patients planning to receive immunosuppressant therapy. Patients found to be positive for anti-HBc, particularly patients who are negative for anti-HBs, should be closely monitored with HBsAg, HBV DNA and serum biochemistry during chemotherapy and for at least 6 mo after the completion of therapy. Further prospective multicenter studies are needed to explore the incidence and risk factors of HBV reactivation in Egypt. Further studies are recommended to determine whether specific genomic mutations are implicated in de novo hepatitis in this subset of patients infected with HBV genotype D1.

COMMENTS

Background

The reactivation of hepatitis B is a syndrome characterized by an abrupt appearance or rise of the hepatitis B virus (HBV) DNA in the sera of patients with resolved or inactive hepatitis B infection. Reactivation can be spontaneous but is typically triggered by cancer chemotherapy, immune suppression or alterations in immune system function. Hepatitis B reactivation is of special clinical concern in immunocompromised patients because it leads to severe liver dysfunction and hepatic failure. However, hepatitis B reactivation is easy to prevent by introducing a prophylactic oral antiviral therapy. Occult hepatitis B is defined by the presence of HBV DNA in the serum or the liver in the absence of Hepatitis B surface antigen (HBsAg) with or without hepatitis B core antibody (anti-HBc) or antibodies to HBV surface antigen (anti-HBs). These patients are at risk of developing hepatitis B reactivation due to any factor suppressing the immune system. In Egypt, patients receiving cancer chemotherapy are typically monitored by liver function tests, with no screening for HBsAg or HBV DNA except in cases with elevated liver enzymes. This study aimed to investigate the incidence of HBV reactivation and the underlying risk factors of reactivation in Egyptian patients with hematological malignancies who were receiving cancer chemotherapy.

Research frontiers

In a cohort of 53 patients with hematological malignancies receiving cancer chemotherapy who were negative for HBsAg, 18 patients (34%) were found to be positive for the anti-HBc, and five of the 53 (9.4%) patients with hematologic malignancies experienced HBV reactivation. All five patients were positive for anti-HBc. HBV DNA was detected in pretreatment HBsAg-negative samples in one of the five patients with HBV reactivation. In this patient, sera were obtained before the start of chemotherapy and at the time of *de novo* HBV hepatitis; the molecular evolutionary analysis of the sequences encompassing the HBV full genome obtained from the sera revealed that this patient developed reactivation from an occult HBV infection.

Innovations and breakthroughs

This study is the first in Egypt to characterize HBV reactivation in Egypt. The study introduces more evidence through molecular evolutionary analysis that occult HBV infection is a risk factor for reactivation of hepatitis B in patients with hematological malignancies receiving cancer chemotherapy.

Applications

The study strongly recommends mandatory serological screening for anti-HBc and anti-HBs in this subset of patients before the commencement of chemotherapy. Patients found to be positive for anti-HBc, particularly patients who are negative for anti-HBs, should be closely observed for signs of HBV reactivation through the regular monitoring of HBsAg and HBV DNA.

Peer review

In the study, performance of sequencing and molecular analysis of HBV genomes seems relevant in characterization of the strains associated with HBV reactivation. Their findings are significant and beneficial for the readers.

REFERENCES

1 **Lee WM**. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745 [PMID: 9392700 DOI: 10.1056/NEJM199712113372406]

2 **Freudiger H**, Sitavanc R. Reverse seroconversion of hepatitis B in a haemodialysis patient. *Nephrol Dial Transplant* 2004; **19**: 238-241 [PMID: 14671065 DOI: 10.1093/ndt/gfg448]

3 **Schnepf N**, Sellier P, Bendenoun M, Zini JM, Sanson-le Pors MJ, Mazeron MC. Reactivation of lamivudine-resistant occult hepatitis B in an HIV-infected patient undergoing cytotoxic chemotherapy. *J Clin Virol* 2007; **39**: 48-50 [PMID: 17368969 DOI: 10.1016/j.jcv.2007.01.011]

4 **Chakvetadze C**, Bani-Sadr F, Le Pendeven C, Lamontagne F, Vincensini JP, Pialoux G. Reactivation of hepatitis B virus

replication during peginterferon-ribavirin therapy in an HIV/hepatitis C virus-co-infected patient with isolated anti-hepatitis B core antibodies. *AIDS* 2007; **21**: 393-394 [PMID: 17255759 DOI: 10.1097/QAD.0b013e328012b5d3]

5 **Clark SJ**, Creighton S, Horner M, Smith HM, Portmann B, Taylor C, Cramp ME. Reactivation of latent hepatitis B virus infection with HIV-related immunosuppression. *Int J STD AIDS* 2006; **17**: 67-69 [PMID: 16409685 DOI: 10.1258/095646206775220612]

6 **Bortolotti F**, Crivellaro C, Brunetto MR, Cadrobbi P, Bertolini A, Alberti A. Selection of a precore mutant of hepatitis B virus and reactivation of chronic hepatitis B acquired in childhood. *J Pediatr* 1993; **123**: 583-585 [PMID: 8410512 DOI: 10.1016/S0022-3476(05)80956-4]

7 **Lowell JA**, Howard TK, White HM, Shenoy S, Huettner PC, Brennan DC, Peters MG. Serological evidence of past hepatitis B infection in liver donor and hepatitis B infection in liver allograft. *Lancet* 1995; **345**: 1084-1085 [PMID: 7715341 DOI: 10.1016/S0140-6736(95)90819-6]

8 **Nordbø SA**, Skaug K, Holter E, Waage A, Brinch L. Reactivation of hepatitis B virus infection in an anti-HBc and anti-HBs positive patient after allogeneic bone marrow transplantation. *Eur J Haematol* 2000; **65**: 86-87 [PMID: 10914949 DOI: 10.1034/j.1600-0609.2000.9l228.x]

9 **Chamorro AJ**, Casado JL, Bellido D, Moreno S. Reactivation of hepatitis B in an HIV-infected patient with antibodies against hepatitis B core antigen as the only serological marker. *Eur J Clin Microbiol Infect Dis* 2005; **24**: 492-494 [PMID: 15990987 DOI: 10.1007/s10096-005-1355-1]

10 **Kitano K**, Kobayashi H, Hanamura M, Furuta K, Ueno M, Rokuhara A, Tanaka E, Umemura T, Kiyosawa K. Fulminant hepatitis after allogenic bone marrow transplantation caused by reactivation of hepatitis B virus with gene mutations in the core promotor region. *Eur J Haematol* 2006; **77**: 255-258 [PMID: 16923112 DOI: 10.1111/j.1600-0609.2006.00678.x]

11 **Lin PC**, Poh SB, Lee MY, Hsiao LT, Chen PM, Chiou TJ. Fatal fulminant hepatitis B after withdrawal of prophylactic lamivudine in hematopoietic stem cell transplantation patients. *Int J Hematol* 2005; **81**: 349-351 [PMID: 15914368 DOI: 10.1532/IJH97.A10411]

12 **Hu KQ**. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat* 2002; **9**: 243-257 [PMID: 12081601 DOI: 10.1046/j.1365-2893.2002.00344.x]

13 **Ozaslan E**, Purnak T. Controversies about occult hepatitis B virus infection. *World J Gastroenterol* 2009; **15**: 4986-4987 [PMID: 19842236 DOI: 10.3748/wjg.15.4986]

14 **Raimondo G**, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007; **46**: 160-170 [PMID: 17112622 DOI: 10.1016/j.jhep.2006.10.007]

15 **Sugiyama M**, Tanaka Y, Kurbanov F, Maruyama I, Shimada T, Takahashi S, Shirai T, Hino K, Sakaida I, Mizokami M. Direct cytopathic effects of particular hepatitis B virus genotypes in severe combined immunodeficiency transgenic with urokinase-type plasminogen activator mouse with human hepatocytes. *Gastroenterology* 2009; **136**: 652-662.e3 [PMID: 19041311 DOI: 10.1053/j.gastro.2008.10.048]

16 **Sugauchi F**, Orito E, Ichida T, Kato H, Sakugawa H, Kakumu S, Ishida T, Chutaputti A, Lai CL, Ueda R, Miyakawa Y, Mizokami M. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *J Virol* 2002; **76**: 5985-5992 [PMID: 12021331 DOI: 10.1128/JVI.76.12.5985-5992.2002]

17 **Shin-I T**, Tanaka Y, Tateno Y, Mizokami M. Development and public release of a comprehensive hepatitis virus database. *Hepatol Res* 2008; **38**: 234-243 [PMID: 17877727 DOI: 10.1111/j.1872-034X.2007.00262.x]

18 **Hui CK**, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, Leung N, Luk JM, Lie AK, Kwong YL, Liang R, Lau GK. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59-68 [PMID: 16831590 DOI: 10.1053/j.gastro.2006.04.015]

19 **Matsue K**, Kimura S, Takanashi Y, Iwama K, Fujiwara H, Yamakura M, Takeuchi M. Reactivation of hepatitis B virus after rituximab-containing treatment in patients with CD20-positive B-cell lymphoma. *Cancer* 2010; **116**: 4769-4776 [PMID: 20597091 DOI: 10.1002/cncr.25253]

20 **Francisci D**, Falcinelli F, Schiaroli E, Capponi M, Belfiori B, Cecchini E, Baldelli F. Reactivation of hepatitis B virus replication due to cytotoxic therapy: a five-year prospective study. *Tumori* 2012; **98**: 220-224 [PMID: 22677988]

21 **Chu CM**, Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. *J Hepatol* 2005; **43**: 411-417 [PMID: 16006001 DOI: 10.1016/j.jhep.2005.03.018]

22 **Romero M**, Madejón A, Fernández-Rodríguez C, García-Samaniego J. Clinical significance of occult hepatitis B virus infection. *World J Gastroenterol* 2011; **17**: 1549-1552 [PMID: 21472119 DOI: 10.3748/wjg.v17.i12.1549]

23 **Said ZN**. An overview of occult hepatitis B virus infection. *World J Gastroenterol* 2011; **17**: 1927-1938 [PMID: 21528070 DOI: 10.3748/wjg.v17.i15.1927]

24 **Samal J**, Kandpal M, Vivekanandan P. Molecular mechanisms underlying occult hepatitis B virus infection. *Clin Microbiol Rev* 2012; **25**: 142-163 [PMID: 22232374 DOI: 10.1128/CMR.00018-11]

25 **Zaaijer HL**, Torres P, Ontañón A, Ponte LG, Koppelman MH, Lelie PN, Hemert FJ, Boot HJ. Multiple surface antigen mutations in five blood donors with occult hepatitis B virus infection. *J Med Virol* 2008; **80**: 1344-1349 [PMID: 18551607 DOI: 10.1002/jmv.21233]

26 **Henke-Gendo C**, Amini-Bavil-Olyaee S, Challapalli D, Trautwein C, Deppe H, Schulz TF, Heim A, Tacke F. Symptomatic hepatitis B virus (HBV) reactivation despite reduced viral fitness is associated with HBV test and immune escape mutations in an HIV-coinfected patient. *J Infect Dis* 2008; **198**: 1620-1624 [PMID: 18847320 DOI: 10.1086/592987]

27 **Sugauchi F**, Tanaka Y, Kusumoto S, Matsuura K, Sugiyama M, Kurbanov F, Ueda R, Mizokami M. Virological and clinical characteristics on reactivation of occult hepatitis B in patients with hematological malignancy. *J Med Virol* 2011; **83**: 412-418 [PMID: 21264861 DOI: 10.1002/jmv.21995]

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BRIEF ARTICLE

Grasper type scissors for endoscopic submucosal dissection of gastric epithelial neoplasia

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Abstract

AIM: To evaluate the efficacy and safety of grasper type scissors (GTS) for endoscopic submucosal dissection (ESD) of gastric epithelial neoplasia.

METHODS: The study was performed by 4 endoscopists in 4 institutions affiliated to The Catholic University of Korea. ESD was performed in 76 consecutive patients with gastric epithelial neoplasia by using the GTS (37 patients) or the hook knife plus coagrasper (HKC) (39 patients). The complete resection rate, complication rate, total time elapsed and elapsed time per square centimeter of the dissected specimen were analyzed between the GTS and HKC group.

RESULTS: The mean age of the GTS group was 62.3 ± 11.4 years and mean age of the HKC group was 65.6 ± 10.1 years. Differentiated adenocarcinoma was found in 32.4% in the GTS group and 33.3% in the HKC group. The procedures were performed without interruption in every case in both groups. The *en bloc* resection rates of both groups were 100%. The total time elapsed during the procedure was 44.54 ± 21.72 min in the GTS group and 43.77 ± 21.84 min in the HKC group (P = 0.88) and the time elapsed per square centimeter of the resected lesion was 7.53 ± 6.35 min/cm^2 in the GTS group and 6.92 ± 5.93 min/cm^2 in the HKC group (P = 0.66). The overall complication rate was not significantly different between the two groups.

CONCLUSION: GTS is a safe and effective device for ESD compared with HKC. ESD can be performed with GTS alone, which can reduce the costs for ESD.

Key words: Gastric epithelial neoplasia; Endoscopic submucosal dissection; Grasper type scissors; Hook knife; Coagrasper

Core tip: Many types of knives have been developed and used for endoscopic submucosal dissection (ESD). We modified the grasping type scissors forceps and developed a novel grasper type scissors (GTS). The aim of this study was to evaluate the efficacy and safety of GTS compared to hook knife plus coagrasper (HKC) for ESD of gastric epithelial neoplasia. The procedures were performed without interruption in every case in both groups. GTS is a safe and effective device for ESD of gastric epithelial neoplasia compared with HKC. ESD can be performed with GTS alone, which can reduce the costs for ESD.

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INTRODUCTION

Endoscopic mucosal resection (EMR) is an endoscopic technique developed for removal of sessile or flat neoplasms confined to the superficial layers (mucosa and submucosa) of the gastrointestinal tract. EMR is typically used for removal of lesions smaller than 20 mm. *En bloc* resection of lesions larger than 20 mm is difficult by EMR thus increasing the risk of local recurrence[1-4]. Newly developed endoscopic techniques and devices have helped to overcome the limitations of EMR in terms of lesion size, location, and presence of fibrotic scarring.

Endoscopic submucosal dissection (ESD) allows *en bloc* resection of larger (usually more than 20 mm) lesions as well as subepithelial gastrointestinal lesions[5]. Various cutting devices - insulated tipped (IT) knife, hook knife, flex knife, triangular knife, and fork knife and so on-have been developed. Grasping-type scissors forceps (GSF) have a 0.8-mm-wide and 6-mm-long serrated cutting edge to facilitate the grasping of tissues. The outer side of the forceps is insulated and the forceps are able to rotate to the desired location. GSF can be used for excision by accurately gripping the submucosal tissue of the target lesion[6-8]. This device has been used safely and effectively for ESD in other organs such as the colorectum and duodenum[9-12]. However, it was not designed for cutting tissues and sometimes it was not optimal when used for dissecting lesions. Furthermore, rotating the GSF to the desired location is frequently difficult.

The newly developed grasper type scissors (GTS) can grasp and cut a piece of tissue using an electrosurgical current. Unlike GSF, the tip of the knife is not insulated and has a thin cutting blade to facilitate submucosal dissection. Theoretically, it possesses both advantages of the GSF and the flex knife.

The aim of this study was to evaluate the efficacy and safety of the novel GTS knife, which can be used for both dissection and hemostasis, for ESD of gastric epithelial neoplasia and compare it to hook knife plus coagrasper (HKC), which is one of the most commonly used knives in South Korea.

MATERIALS AND METHODS

Ethical consideration

The advantages and disadvantages of ESD with GTS, as well as alternative endoscopic options (*e.g.*, ESD with a conventional device, EMR) were discussed with each patients. All patients gave their written informed consent to the designated intervention. This study protocol was approved by the Institutional Review Board of The Catholic University of Korea.

Patients

Patients with gastric epithelial neoplasia were consecutively enrolled between May 2010 and April 2012. The study was a prospective, randomized, multi-center, comparative trial. It was performed by 4 endoscopists in 4 institutions affiliated with The Catholic University of Korea (Incheon St. Mary's Hospital, St. Vincent's Hospital, Seoul St. Mary's Hospital, and Bucheon St. Mary's Hospital). All of the endoscopists were experienced with ESD and had performed ESD in over 200 cases. Before this study, two endoscopists (Kim BW, Lim CH) had used hook knife as the main device and the other two endoscopists (Chung WC, Kim TH) had used flex knife as the main device.

Adults (> 18 years) with histopathologic diagnosis of gastric epithelial neoplasia and without evidence of lymph-node involvement documented by abdominal computed tomography (CT) and/or endoscopic ultrasound (EUS) were included in this study. The lesions met the expanded criteria for local resection proposed by Gotoda[5,13,14]. Differentiated mucosal cancers of any size without ulceration or scarring, differentiated mucosal cancers < 30 mm in diameter with ulceration or scarring, or differentiated cancers with minimal submucosal invasion (< 500 μm deep in the submucosa starting from the muscularis mucosae) were enrolled. The diameter of the lesions without ulcers was limited to a maximum of 60 mm. Patients with conditions that might have substantial effects on our study results (*e.g.*, serum creatinine > 2.5 mg/dL, total bilirubin > 3.0 mg/dL, platelet < 100000/mm^3), patients who were consuming anti-platelet agents, patients with a history of previous gastric surgery and patients who did not consent to the study were excluded.

Sample size

An estimated sample size of 37 subjects per group would give an 80% power to detect a difference in resection rate of the GTS compared to the HKC (assumed to have a complete resection rate of 90%), with a two-sided α = 0.05. With a 10% drop out rate, 40 patients had to be recruited for each group.

Randomization

All patients were randomly assigned to receive one of the knives-hook knife (Olympus; Tokyo, Japan) plus coagrasper (Olympus; Tokyo, Japan) or grasper type scissors (Alton Medical Instruments; Shanghai, China) (Figure 1) after evaluation with abdominal CT and/or EUS. Randomization codes (A-1 to A-10, B-1 to B-10) were packed into sealed opaque envelopes by an individual, who was not involved in screening and enrolment of the subjects to ensure concealment of allocation. In each of the study institute, twenty patients were enrolled.

Figure 1 Distal tip of the grasper type scissors. GTS has teeth inside the device and the outer side is not insulated. GTS: Grasper type scissors.

Grasper type scissors

The diameter of the scissors is 2.4 mm and the serrated cutting edges are 4-mm-long. The outer side of the forceps except the tip is insulated so that electrosurgical current energy is concentrated at the blade to avoid burning the surrounding tissue. Unlike GSF, the tip of this knife is not insulated and has a thin cutting blade, so that it can facilitate the dissection of submucosal layer such as a flex knife. Furthermore, the forceps can be rotated to the desired location.

Endoscopic submucosal dissection

A conventional gastroscope (GIF-Q240J or GIF-H260Z; Olympus, Tokyo, Japan) fitted with a transparent distal attachment (D-201-11304, Olympus) was used for the ESD procedures regardless of the ESD devices. Patients were sedated with intravenous midazolam (0.1 mg/kg) while in the endoscopic suite, and conscious sedation was maintained with additional injections during the procedure. After spraying indigo carmine dye, circumferential markings using argon plasma coagulation were made at 5 mm distances around the outside margin of the lesion, with 2 mm intervals between each marking dot. Hypertonic saline/epinephrine solution (1:10000) and indigo carmine mixture was injected into the submucosal layer until the mucosa was raised and additional injections were repeated as necessary during the procedure. After the lesion was lifted, mucosal incision was performed by using each type of knife and an electrosurgical generator (Erbe; Tübingen, Germany). Electrical current was set as endocut- I for hook knife and as endocut-Q for GTS. After incision around the lesion, dissection was conducted with either the hook knife or GTS (Figure 2).

Electrical current was set as forced coagulation for hook knife and as swift coagulation for GTS. When bleeding occurred during dissection, saline irrigation was performed. If the endoscopist performed the procedure with a hook knife, coagrasper was used for hemostasis according to the endoscopists' instructions, with an electrical current of 80 W for soft coagulation. With GTS, hemostasis was performed with an electrical current of 80 W for soft coagulation mode.

Measurements

We compared the endoscopic appearance of tumors, location of tumors, *en bloc* resection rate, complete resection rate, size of resected specimens, histopathologic findings, operation time, and complication rates between the two groups. Complete resection was defined as lateral and vertical margins of the specimen being free from tumor involvement. Marking of the first dot and withdrawal of the endoscope were measured as the procedure time.

Bleeding was identified when melena, hematochezia, or the presence of fresh bloody vomitus along with a decreased hemoglobin levels of more than 2 g/dL were present after the resection. Perforation was identified by endoscopy during or just after the procedure and/or by the presence of intraperitoneal free air on plain abdominal radiography after the procedure.

Statistical analysis

All data were recorded on standard forms and computer analyzed. The Student *t* test was used to compare continuous variables between the groups. Differences between dichotomous variables were evaluated with the χ^2 test. The calculations were performed with the SPSS software (SPSS version 12.0, Chicago, IL, United States). Null hypotheses of no difference were rejected if *P* values were less than 0.05.

RESULTS

A total of 78 patients were enrolled and 76 patients (37 patients of GTS knife group and 39 patients with HKC group) completed this study. Since over 37 patients in each group completed this protocol, additional enrollment was not conducted. One patient dropped out of the study because undifferentiated cancer was found in the resected specimen. Another patient with extensive submucosal infiltration (> 500 μm deep in the submucosa starting from the muscularis mucosae) was also dropped out of the study. Both patients underwent additional surgery after ESD.

The mean age of the GTS group was 62.3 ± 11.4 years and mean age of the HKC group was 65.6 ± 10.1 years (Table 1).There was no significant difference in age and sex ratio between the two groups. Differentiated adenocarcinoma was found in 32.4% in the GTS group and 33.3% in the HKC group. The pathologic distribution and mean size of the resected specimens were not different between the two groups. The area of the resected specimens was 8.30 ± 4.52 cm^2 in GTS group and 8.59 ± 4.43 cm^2 in HKC group. The depth of tumor distribution was not different between the two groups (94.6% of mucosal layer in the GTS group, 92.3% in the HKC group). With regard to tumor location, 64.9% (24/37 cases) were located at the antrum in the GTS group and 71.8% (28/39 cases) in the HKC group. The locations were not significantly different between the two groups. The total time

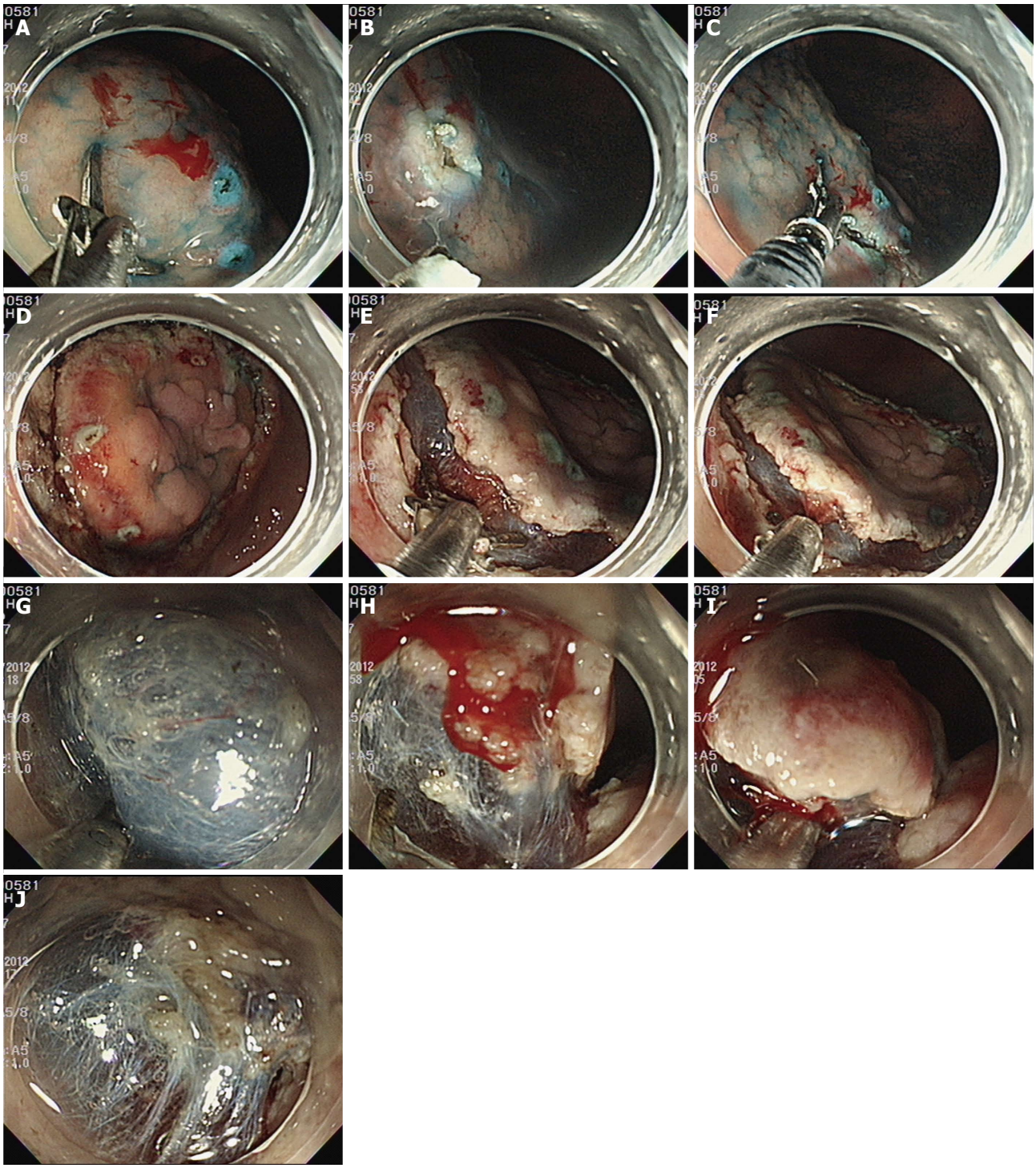

Figure 2 Procedure of endoscopic submucosal dissection with distal tip of the grasper type scissors. A: Grasper type scissors (GTS) is ready for puncture; B: Puncture was completed with GTS; C: Incision is conducted with GTS; D: Complete incision was performed with GTS; E: Submucosal dissection is ready with GTS; F: Submucosal dissection is conducted by grasping the submucosal layer; G: Submucosal dissection is conducted with blade of the GTS just like a flex knife; H: Hemorrhage from the submucosal layer is noted; I: Bleeding focus was grasped with GTS; J: Bleeding was controlled after coagulation with GTS.

elapsed during the procedure was 44.54 ± 21.72 min in the GTS group and 43.77 ± 21.84 min in the HKC group (P = 0.88). The time elapsed per square centimeter of the resected lesion was 7.53 ± 6.35 min/cm^2 in the GTS group and 6.92 ± 5.93 min/cm^2 in the HKC group (P = 0.66). The *en bloc* resection rate was 100% in both groups. The overall complication rate was 5.41% (2/37 cases) in the GTS group and 7.69% (3/39 cases) in the HKC group (P = 0.68).

In Korea, the prices of the knives used for ESD are the same. GTS and hook knife cost about 199240 Won (about 185 dollars) and coagrasper cost about 210000 Won (about 191 dollars). HKC are about double in cost compared to GTS alone.

Table 1 Characteristics of the enrolled patients *n* (%)

Characteristics	GTS	HKC	*P* value
Number of patients (total)	37	39	0.30
Operator 1 (Kim BW)	10	10	
Operator 2 (Chung WC)	9	10	
Operator 3 (Lim CH)	8	9	
Operator 4 (Kim TH)	10	10	
Male:female	26:11	23:16	0.19
Age (mean ± SD, yr)	62.3 ± 11.4	65.6 ± 10.1	0.58
Final pathologic diagnosis			0.68
Tubular adenoma, low grade dysplasia	22	20	
Tubular adenoma, high grade dysplasia	3	6	
Adenocarcinoma, well differentiated	6	8	
Adenocarcinoma, moderately differentiated	6	5	
Location of the lesion			
Antrum	24	28	
Angle	5	3	
Body	8	8	
Long axis (cm)	3.59 ± 1.10	3.68 ± 0.98	0.70
Short axis (cm)	2.75 ± 0.83	2.79 ± 0.84	0.83
Area (cm^2)	8.30 ± 4.52	8.59 ± 4.43	0.78
Total elapsed time (mean ± SD, min)	44.54 ± 21.72	43.77 ± 21.84	0.88
Elapsed time/cm^2 (mean ± SD, min/cm^2)	7.53 ± 6.35	6.92 ± 5.93	0.66
En bloc resection	37/37 (100)	39/39 (100)	
Incomplete resection	0/37 (0)	0/39 (0)	
Complications	1/37 (2.7)	2/39 (5.1)	
Perforation	1	1	
> 2.0 g/dL Hb decrease 1 d after procedure	0	1	

GTS: Grasper type scissors; HKC: Hook knife plus coagrasper.

DISCUSSION

Conventional EMR was previously recommended as a curative local treatment for early gastric cancer[15,16]. To achieve curative resection with adequate margins, the excisions need to be large enough. When piecemeal resection occurs, electro-cautery across the specimens could affect the accuracy of assessment of the lateral margins and this could result in a higher risk of local recurrence. ESD is intended to perform large mucosal resections and improves the rate of successful *en bloc* resection of an early stage gastrointestinal neoplasia[17,18]. To date, novel devices have been developed for the completion of *en bloc* resection with adequate margins. However, many endoscopists are eager for the development of devices that allow more effective and faster procedures. In this study, we aimed to introduce and to evaluate the efficacy and technical aspects of the GTS.

Since GSF is ideally designed for both incising the targeted tissue and hemostasis, we tried to improve the device by improving its effect during dissection. Theoretically, the advantage of GSF for ESD is that the device can prevent unexpected incisions. By elevating the lesion during dissection, GSF provides good visualization of the submucosal layer. However, GSF has the disadvantage that it cannot be opened when using a conventional cap because of the small cap diameter. Therefore, a special hood is required when using the GSF. We modified the GSF and developed the GTS, which can accurately grasp and incise the targeted tissue using electrosurgical current. It is smaller than GFS so that it can be used with the conventional transparent cap. The most important difference between GTS and GSF is that the tip of GTS is not insulated and has a thin cutting blade, so that it can facilitate the dissection of the submucosal layer.

Most of the knives are designed for cutting or dissection, but they are not adequate for hemostasis during the ESD procedure. The use of additional instruments increases the cost and requires more procedure time because it takes time to change the instruments. The scissor-type device makes it possible to perform dissection and hemostatic procedures without changing the devices. In this study, we compared the novel GTS, which can be used for both dissection and hemostasis, with HKC for ESD of gastric epithelial neoplasia. We compared these knives because HKC is one of the most commonly used devices for ESD in Korea. In our results, the elapsed time did not differ significantly between GTS and HKC and the procedure time was not saved by using the GTS. This result may have been caused by various factors such as the fact that the endoscopists of this study were not experienced with the new instrument. Furthermore, 2 of the endoscopists had been using the HKC and were familiar with this device which may have affected the procedure time. It is known that there is a learning curve for ESD and that experience of the procedure shortens the procedure time[19,20]. We believe that increased experience with the GTS will reduce the procedure time. Nonetheless all the lesions were removed successfully with the GTS by the 4 endoscopists, which suggest that any experienced endoscopists can complete the whole ESD procedure with the GTS. Feasibility of ESD for gastric epithelial neoplasia with the GTS in beginners should be elucidated in the future.

One case of perforation occurred in the GTS group which might have been prevented if a knife with an insulated body such as GSF was used. The perforation rates of ESD while using different types of knifes were reported to be 4%-10%[21-24], which is similar to the perforation rates of our result. A decrease in hemoglobin level over 2 g/dL per day after the procedure was the same in both groups. Thermal and mechanical tissue damage at the GTS-tissue interface was expected because GTS is larger compared to the hook knife. However, contrary to our expectations, GTS did not interfere with the pathologists' interpretation of the specimens and complete resections with adequate margins were obtained pathologically with GTS.

There are some limitations in this study. We designed this device to reduce the procedure time, but the sample size was limited and we could not figure it out. Four experienced endoscopits participated in this study and the

procedure times by beginners also should be examined. Further studies with larger sample sizes are anticipated to show other benefits of this device.

In conclusion, ESD for gastric epithelial neoplasia can be performed with GTS alone regardless of size and location of the lesion when the endoscopists are experienced. GTS is a safe and effective device for ESD of gastric epithelial neoplasia when compared to HKC. ESD can be performed with GTS alone, which can reduce the costs. Further experiences and large scaled studies would be anticipated to compare the various devices.

COMMENTS

Background

Various cutting devices have been developed for endoscopic submucosal dissection (ESD). Most of these knives are designed for dissection of the submucosa and sometimes are not inadequate for control hemorrhages developed during the procedure. The authors recently designed a new device, grasper type scissors (GTS) both for dissection and control hemorrhages.

Research frontiers

Grasping-type scissors forceps (GSF) was developed by Akahoshi *et al* for dissection and control hemorrhages. However, it requires a special hood and rotating the device is frequently difficult because of the size. The safety and effectiveness of GSF was performed in a single center.

Innovations and breakthroughs

The most important difference between GTS and GSF is that the tip of GTS is not insulated and has a thin cutting blade, so that it can facilitate the dissection of the submucosal layer. The safety and effectiveness of GTS was evaluated in multi-centers in this study.

Applications

This study suggests that ESD for gastric epithelial neoplasia can be performed with GTS alone regardless of size and location of the lesion when the endoscopists are experienced.

Peer review

The authors developed a novel device which was modified from GSF, GTS, for ESD of gastric neoplasia. In this study, GTS resolved several disadvantage of GSF and clearly succeeded to reduce the cost of ESD. This study is very interesting, exciting and useful for all endoscopists.

REFERENCES

1 **Uedo N**, Iishi H, Tatsuta M, Ishihara R, Higashino K, Takeuchi Y, Imanaka K, Yamada T, Yamamoto S, Yamamoto S, Tsukuma H, Ishiguro S. Longterm outcomes after endoscopic mucosal resection for early gastric cancer. *Gastric Cancer* 2006; **9**: 88-92 [PMID: 16767363]

2 **Muto M**, Miyamoto S, Hosokawa A, Doi T, Ohtsu A, Yoshida S, Endo Y, Hosokawa K, Saito D, Shim CS, Gossner L. Endoscopic mucosal resection in the stomach using the insulated-tip needle-knife. *Endoscopy* 2005; **37**: 178-182 [PMID: 15692936]

3 **Kim JJ**, Lee JH, Jung HY, Lee GH, Cho JY, Ryu CB, Chun HJ, Park JJ, Lee WS, Kim HS, Chung MG, Moon JS, Choi SR, Song GA, Jeong HY, Jee SR, Seol SY, Yoon YB. EMR for early gastric cancer in Korea: a multicenter retrospective study. *Gastrointest Endosc* 2007; **66**: 693-700 [PMID: 17905010]

4 **Hulagu S**, Senturk O, Aygun C, Kocaman O, Celebi A, Konduk T, Koc D, Sirin G, Korkmaz U, Duman AE, Bozkurt N, Dindar G, Attila T, Gurbuz Y, Tarcin O, Kalayci C. Endoscopic submucosal dissection for premalignant lesions and noninvasive early gastrointestinal cancers. *World J Gastroenterol* 2011; **17**: 1701-1709 [PMID: 21483630 DOI: 10.3748/wjg.v17.i13.1701]

5 **Syrén PO**, Hult K. Substrate conformations set the rate of enzymatic acrylation by lipases. *Chembiochem* 2010; **11**: 802-810 [PMID: 20301160 DOI: 10.1002/bjs.7033]

6 **Akahoshi K**, Akahane H, Murata A, Akiba H, Oya M. Endoscopic submucosal dissection using a novel grasping type scissors forceps. *Endoscopy* 2007; **39**: 1103-1105 [PMID: 18072064]

7 **Akahoshi K**, Honda K, Akahane H, Akiba H, Matsui N, Motomura Y, Kubokawa M, Endo S, Higuchi N, Oya M. Endoscopic submucosal dissection by using a grasping-type scissors forceps: a preliminary clinical study (with video). *Gastrointest Endosc* 2008; **67**: 1128-1133 [PMID: 18355820 DOI: 10.1016/j.gie.2007.12.007]

8 **Uraoka T**, Kawahara Y, Ohara N, Kato J, Hori K, Okada H, Yamamoto K. Carbon dioxide submucosal injection cushion: an innovative technique in endoscopic submucosal dissection. *Dig Endosc* 2011; **23**: 5-9 [PMID: 21198910 DOI: 10.1111/j.1443-1661.2010.01037.x]

9 **Akahoshi K**, Okamoto R, Akahane H, Motomura Y, Kubokawa M, Osoegawa T, Nakama N, Chaen T, Oya M, Nakamura K. Endoscopic submucosal dissection of early colorectal tumors using a grasping-type scissors forceps: a preliminary clinical study. *Endoscopy* 2010; **42**: 419-422 [PMID: 20340070 DOI: 10.1055/s-0029-1243973]

10 **Akahoshi K**, Honda K, Kubokawa M, Motomura Y, Matsui N, Endo S, Higuchi N, Taki K, Oya M, Akahane H, Akiba H. Endoscopic resection of a large pedunculated duodenal polyp using a grasping type scissors forceps. *Endoscopy* 2008; **40** Suppl 2: E74-E75 [PMID: 18633904 DOI: 10.1055/s-2007-995524]

11 **Akahoshi K**, Akahane H. A new breakthrough: ESD using a newly developed grasping type scissor forceps for early gastrointestinal tract neoplasms. *World J Gastrointest Endosc* 2010; **2**: 90-96 [PMID: 21160708 DOI: 10.4253/wjge.v2.i3.90]

12 **Akahoshi K**, Motomura Y, Kubokawa M, Matsui N, Oda M, Okamoto R, Endo S, Higuchi N, Kashiwabara Y, Oya M, Akahane H, Akiba H. Endoscopic submucosal dissection of a rectal carcinoid tumor using grasping type scissors forceps. *World J Gastroenterol* 2009; **15**: 2162-2165 [PMID: 19418591]

13 **Gotoda T**, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225 [PMID: 11984739]

14 **Gotoda T**. A large endoscopic resection by endoscopic submucosal dissection procedure for early gastric cancer. *Clin Gastroenterol Hepatol* 2005; **3**: S71-S73 [PMID: 16013003]

15 **Adachi Y**, Shiraishi N, Kitano S. Modern treatment of early gastric cancer: review of the Japanese experience. *Dig Surg* 2002; **19**: 333-339 [PMID: 12435900]

16 **Soetikno R**, Kaltenbach T, Yeh R, Gotoda T. Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract. *J Clin Oncol* 2005; **23**: 4490-4498 [PMID: 16002839]

17 **Lian J**, Chen S, Zhang Y, Qiu F. A meta-analysis of endoscopic submucosal dissection and EMR for early gastric cancer. *Gastrointest Endosc* 2012; **76**: 763-770 [PMID: 22884100 DOI: 10.1016/j.gie.2012.06.014]

18 **Park YM**, Cho E, Kang HY, Kim JM. The effectiveness and safety of endoscopic submucosal dissection compared with endoscopic mucosal resection for early gastric cancer: a systematic review and metaanalysis. *Surg Endosc* 2011; **25**: 2666-2677 [PMID: 21424201 DOI: 10.1007/s00464-011-1627-z]

19 **Kato M**, Gromski M, Jung Y, Chuttani R, Matthes K. The learning curve for endoscopic submucosal dissection in an established experimental setting. *Surg Endosc* 2013; **27**: 154-161 [PMID: 22806508 DOI: 10.1007/s00464-012-2402-5]

20 **Yamamoto S**, Uedo N, Ishihara R, Kajimoto N, Ogiyama H, Fukushima Y, Yamamoto S, Takeuchi Y, Higashino K, Iishi H, Tatsuta M. Endoscopic submucosal dissection for early gastric cancer performed by supervised residents: assessment of feasibility and learning curve. *Endoscopy* 2009; **41**: 923-928

[PMID: 19802773 DOI: 10.1055/s-0029-1215129]
21 **Kato M**. Endoscopic submucosal dissection (ESD) is being accepted as a new procedure of endoscopic treatment of early gastric cancer. *Intern Med* 2005; **44**: 85-86 [PMID: 15750264]
22 **Oka S**, Tanaka S, Kaneko I, Mouri R, Hirata M, Kawamura T, Yoshihara M, Chayama K. Advantage of endoscopic submucosal dissection compared with EMR for early gastric cancer. *Gastrointest Endosc* 2006; **64**: 877-883 [PMID: 17140890]
23 **Gotoda T**, Yamamoto H, Soetikno RM. Endoscopic submucosal dissection of early gastric cancer. *J Gastroenterol* 2006; **41**: 929-942 [PMID: 17096062]
24 **Lee H**, Yun WK, Min BH, Lee JH, Rhee PL, Kim KM, Rhee JC, Kim JJ. A feasibility study on the expanded indication for endoscopic submucosal dissection of early gastric cancer. *Surg Endosc* 2011; **25**: 1985-1993 [PMID: 21136092 DOI: 10.1007/s00464-010-1499-7b]

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BRIEF ARTICLE

Isolation and biochemical analysis of vesicles from taurohyodeoxycholic acid-infused isolated perfused rat livers

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Abstract

AIM: To isolate biliary lipid-carrying vesicles from isolated perfused rat livers after taurohyodeoxycholic acid (THDC) infusion. Biliary lipid vesicles have been implicated in hepatic disease and THDC was used since it increases biliary phospholipid secretion.

METHODS: Rat livers were isolated and perfused *via* the hepatic portal vein with THDC dissolved in Krebs Ringer Bicarbonate solution, pH 7.4, containing 1 mmol/L $CaCl_2$, 5 mmol/L glucose, a physiological amino acid mixture, 1% bovine serum albumin and 20% (v/v) washed human erythrocytes at a rate of 2000 nmol/min for 2 h. The livers were then removed, homogenized and subjected to centrifugation, and the microsomal fraction was obtained and further centrifuged at 350000 *g* for 90 min to obtain subcellular fractions. These were analyzed for total phospholipid, cholesterol, protein and alkaline phosphodiesterase I (PDE).

RESULTS: No significant changes were observed in the total phospholipid, cholesterol and protein contents of the gradient fractions obtained from the microsomal preparation. However, the majority of the gradient fractions (ρ= 1.05-1.07 g/mL and ρ = 1.95-1.23 g/mL) obtained from THDC-infused livers had significantly higher PDE activity compared to the control livers. The low density gradient fraction (ρ = 1.05-1.07 g/mL) which was envisaged to contain the putative vesicle population isolated from THDC-perfused livers had relatively small amounts of phospholipids and protein when compared to the relevant control fractions; however, they displayed an increase in cholesterol and PDE activity. The phospholipids were also isolated by thin layer chromatography and subjected to fractionation by high performance liquid chromatography; however, no differences were observed in the pattern of the fatty acid composition of the phospholipids isolated from THDC and control perfused livers. The density gradient fractions (ρ = 1.10-1.23 g/mL) displayed an increase in all the parameters measured from both control and THDC-infused livers.

CONCLUSION: No significant changes in biliary lipids were observed in the fractions from THDC-infused livers; however, PDE activity was significantly increased compared to the control livers.

Key words: Taurohyodeoxycholic acid; Phospholipids; Biliary cholesterol; Bile; Vesicles

Core tip: Bile contains various constituents including cholesterol and phospholipid, mainly phosphatidylcholine with a unique fatty acid composition of 1-palmitoyl 2-linoleyl (16:0-18:2) phosphatidylcholine and 1-palmitoyl 2-oleoyl (16:0-18:1). These biliary lipids are transported in vesicles from a specific intra-hepatic pool and an increase in biliary lipid-carrying vesicles may have

implications for hepatic diseases such as gallstone formation. Taurohyodeoxycholic acid (THDC) stimulates the secretion of biliary phospholipids; hence THDC-infused rat livers were subjected to ultracentrifugation in order to isolate these phospholipid-carrying vesicles. The isolation of these biliary lipid-carrying vesicles was not successful; however, vesicles enriched in PDE activity were obtained.

Hismiogullari AA, Hismiogullari SE, Rahman K. Isolation and biochemical analysis of vesicles from taurohyodeoxycholic acid-infused isolated perfused rat livers. *World J Gastroenterol* 2013; 19(37): 6228-6236 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6228.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6228

INTRODUCTION

Phospholipids and cholesterol are synthesized in the hepatocytes and are thought to be transferred into bile by vesicular and non-vesicular mechanisms. Biliary lipids mainly consist of cholesterol and phospholipids and their secretion into bile is effected by secretion of bile salts[1]. Hepatocytes acquire biliary lipid by three pathways namely biosynthesis, lipoproteins and existing lipid molecules drawn from intracellular membranes and newly synthesized biliary lipids; these account for less than 20% of the total lipids[2].

The majority of biliary phospholipid is phosphatidylcholine (PC) with distinct fatty acid composition, namely 1-palmitoyl 2-linoleyl (16:0-18:2) PC and 1-palmitoyl 2-oleoyl (16:0-18:1), whereas hepatocyte PC contains significant amounts of different phospholipid classes[3,4]. Very few studies have been performed on biliary lipid transport in hepatocytes[5,6], whereas numerous studies have been performed on bile, its formation and composition especially related to lipids, and these studies have identified several physical forms of lipid carriers, including biliary vesicles[7,8].

The main source of biliary lipid, before its appearance in bile, has been suggested to be the bile canalicular membrane where it is removed by the detergent action of bile salts. These lipids are then thought to be continuously replaced from within the cell, probably *via* vesicular transport, for biliary lipid secretion to continue without damage to the liver and canalicular membrane[9]. In support of this, inhibitors of microtubular function such as colchicine and vinblastine have been shown to reduce biliary lipid secretion[10-12]. Such vesicles supplying lipids to the plasma membrane have also been shown and isolated in other cell types, thus, it can be postulated that biliary lipid is probably supplied to the canalicular membrane *via* such vesicles. This is supported by the fact that increased numbers of vesicles have been observed accumulating near the bile canaliculus during extensive bile acid secretion[2,9,13-15]. The isolation of putative biliary lipid-carrying vesicles, however, is difficult due to the wide range of vesicle types in hepatocytes, and the difficulty of identifying them because of inadequate criteria.

Hepatic ATP-binding cassette half-transporter genes 5/8 (*ABCG5* and *ABCG8*) are expressed in the canalicular membrane of hepatocytes and have an essential role in biliary cholesterol secretion[16-19]. However, the pathways involved in trans-hepatic cholesterol trafficking into bile are still not clear and a specific cholesterol transport protein has not been confirmed in hepatocytes. Biliary cholesterol secretion is important for the two important disease complexes of atherosclerotic cardiovascular disease (CVD) and gallstone disease[1]. In atherosclerotic CVD, biliary cholesterol secretion is thought to be the final step in the completion of the reverse cholesterol transport pathway which includes the transport of peripheral cholesterol back to the liver for excretion into bile. Increase in biliary cholesterol secretion can lead to the supersaturation of bile and under the right conditions this may lead to the formation of cholesterol gallstones[1]. Taurohyodeoxycholic acid (THDC) is a natural 6α-hydroxylated bile acid with hydrophilic properties, causing more secretion of PC into bile compared to tauroursodeoxycholic acid and taurocholic acid, whereas no significant differences were found in the biliary secretion of cholesterol[20]. Due to its relatively high hydrophilicity, THDC has been proposed for use instead of other bile acids for the treatment of cholesterol gallstone dissolution[21]. Angelico *et al*[20] showed by the use of electron microscopy that increased recruitment of vesicles and lamellar bodies around and within bile canaliculi in the liver occurred with THDC infusion. The identification of biliary lipid-carrying vesicles may have implications for the treatment of hepatic disorders such as cholesterol gallstone formation.

The aim of this study was to isolate these biliary lipid-carrying vesicles in hepatocytes by using a novel gradient centrifugation technique and to verify their origin by separating PC by thin layer chromatography (TLC) and measuring its unique fatty acid pattern by high-performance liquid chromatography (HPLC). Cholesterol was measured by gas liquid chromatography (GLC).

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma Chemical Co., Poole, Dorset, United Kingdom, except for cannulation tubing PP10 (internal diameter 0.28 mmol/L) which was obtained from Portex Ltd., Hythe, United Kingdom.

Animals and treatments

Animals used throughout this study were male Wistar rats (250-300 g), bred within Liverpool John Moores University, Life Services Support Unit, and they were allowed free access to standard laboratory diet in powdered form.

Perfusion of isolated liver

Rats were anaesthetized with sodium pentobarbitone (6 mg/100 g body weight, intraperitoneally) before starting

the experiment. Once isolated, livers were perfused in the absence and presence of THDC infusion *in situ* using the method of Rahman and Coleman[22]. Heparin (2500 units/0.5 mL) was injected into the vena cava and after 2 min, the hepatic portal vein was cannulated with a Wallace 17.5 G cannula and the perfusion was commenced immediately with 150 mL of Krebs ringer bicarbonate buffer, pH 7.4, containing 1 mmol/L $CaCl_2$, 5 mmol/L glucose, a physiological amino acid mixture, 1% bovine serum albumin and 20% (v/v) washed human erythrocytes, and the abdominal aorta was severed. The inferior vena cava was then cannulated with a Wallace 16 G cannula and a recycling perfusion commenced by returning the efferent perfusate to the original perfusate pool which was gassed continuously with O_2/CO_2 (19:1, v/v). The livers were maintained in a thermostatically controlled cabinet at 37 ℃ throughout the experiment. As soon as the liver perfusion was established, THDC infusion was commenced into the hepatic portal cannula at a rate of 2000 nmol/min for 2 h to stimulate delivery of lipid-carrying vesicles to the canalicular membrane.

Liver homogenization

At the end of perfusion livers were removed, weighed and transferred to 3 vol. (w/v) of ice-cold buffered sucrose (0.25 mol/L containing 1 mmol/L HEPES pH 7.4). They were then cut into several large pieces and swirled around in the buffer to remove as much blood as possible. The livers were then minced finely with sharp scissors, transferred to an ice-cold homogenizing vessel and were finally homogenized with about six strokes of the pestle at full speed. Finally, the homogenate was made up to 4 vol. (w/v) with sucrose buffer solution.

Fractionation of liver homogenate

The homogenate from the liver was used to produce subcellular fractions based on the method of Ford and Graham[23]. A sample of homogenate (3-4 mL) was removed for analysis and the remainder was centrifuged in a fixed angle rotor at 4 ℃ for 10 min at 1000 *g* to pellet the nuclei and heavy mitochondria. The pellet was then suspended in sucrose buffer and stored frozen at -20 ℃ until analysis.

Further centrifugation was performed at 4000 *g* for 10 min to produce the mitochondrial fraction, followed by 15000 *g* for 20 min to produce the light mitochondrial and lysosome fraction. A final centrifugation step at 100000 *g* for 45 min was then performed and the microsomal fraction was obtained. All fractions were assayed for cholesterol, phospholipids, protein and PDE activity

Purification of vesicles from microsomal fraction

The microsomal pellet was then dissolved in sucrose buffer solution up to 8 mL and then loaded onto 2 mL of OptiPrep™ (1.32 g/mL) in a Beckman Vti65 vertical tube rotor and centrifuged at 350000 *g* for 90 min at 4 ℃. At the end of the centrifugation, the gradient was fractioned by upward displacement into 10 × 1 mL samples and these fractions were analyzed for cholesterol, phospholipids, protein and PDE activity.

Lipid analysis

Cholesterol was analyzed by GLC as trimethylsilyl ether derivatives as described by Zak *et al*[24]. Phospholipid was extracted from the liver fractions as described by Bligh and Dyer[25] in a method by which lipid is extracted into a chloroform-methanol-water mixture. Addition of further chloroform and water forms a biphasic system with non-lipids passing into the methanol-water phase. The phospholipid in the chloroform phase was then assayed by the method of Bartlett[26] in which organic phosphate is digested and the resulting orthophosphate is determined by converting it to phosphomolybdic acid, which is reduced to a blue complex allowing spectrophotometric measurement at 830 nm.

Alkaline phosphodiesterase I analysis

PDE (EC 3.1.4.1) was measured at 37 ℃, essentially as described by Trams and Lauter[27].

Thin layer chromatography of phospholipids

Sample extraction: 200 μL of sample was added to 200 μL of distilled water in a 2 mL (microcentrifuge) tube followed by the addition of 750 μL of chloroform then methanol (1:2 v/v) to each tube, vortex mixed and left to stand for 20 min. After this time, 250 μL of chloroform and 250 μL of distilled water were added and the tubes were vortex mixed and then centrifuged for 1 min. The lower organic phase was then transferred to a clean tube and placed in a water bath at 37 ℃ to evaporate the chloroform[25]. Samples were finally redissolved in 30 mL of chloroform, vortex mixed and loaded on to the TLC plates.

Solvent for running phospholipid plates: The plates were developed in a solvent mixture containing chloroform/methanol/glacial acetic acid/water (75:45:12:1.5 by volume). These solvent ratios were poured into a tank containing a filter paper layered against the wall of the chamber and the lid was replaced; the tank was then left to saturate for at least 30 min prior to running the plates. The plates were left to run until solvent reached the scored solvent front line (approximately 75 min) and were then removed and air dried in a fume cupboard.

Developing the TLC plates: The dried plates were developed in an iodine tank and the position of the phospholipid was marked with a needle. The PC bands were scraped and the silica transferred to extraction tubes; at the same time silica was scraped from a similar area without any phospholipids to act as a control. Phospholipids were extracted with 2 × 1 mL methanol (HPLC grade) and vortex mixed for 5 min, vortexed again, centrifuged and the methanol extract was then transferred to clean glass tubes and dried at 37 ℃ under nitrogen. The dried lipids were redissolved in 1 mL of methanol (HPLC grade) and 2 × 10 μL aliquots were removed for phospholipids assay. The remainder was filtered, dried at 37 ℃

under nitrogen and stored cool and in the dark until required for HPLC analysis. At least 20 nmol of PC was injected onto the HPLC column.

HPLC

HPLC analysis was performed using a Bio-Rad HRLC 2700, Series 8000 Gradient System V 2.30.1a liquid chromatograph equipped with an oven column module, and a spectrophotometric detector, Bio-Rad Model 1801 UV monitor. The sample was injected onto the column by a Rheodyne injector equipped with a 20 μL sample loop. An HPLC column of 100 mmol/L × 4.6 mmol/L *id*, packed with a Spherisorb ODS2 bonded phase, and with a 3 μL particle size was used and the mobile phase consisted of 20 mmol/L choline chloride in methanol/water/acetonitrile (90:8:3, by vol.). The operating conditions were: column temperature, 60 ℃; chromatographic profile: initial flow, 1 mL/min, held for min and then a linear increase to 2 mL/min over 20 min; the final flow of 2 mL/min being held for 10 min.

Protein estimation

Protein estimation was determined by the method of Winterbourne and all samples were assayed in duplicate[28]. A sheet of 3 mmol/L Whatman chromatography paper was divided into 1 cm × 1 cm squares. Standard protein concentration BSA ranged from 0.5-8 mg/mL and the standards were prepared by spotting corresponding amounts onto the center of the squares on the sheet. 3 μL of sample was carefully spotted onto the center of individual squares and blank squares were left for the determination of background staining. The standards and samples were then left to dry and were later fixed by immol/Lersing into 10% TCA solution for 15 min and the sheet was then transferred to a working dye solution (0.04% w/v Coomassie Blue, 25% v/v ethanol and 12% v/v acetic acid) and left to stain for 1 h. The sheet was then destained by immersing in three changes of destaining solution 10% (v/v) methanol and 5% (v/v) glacial acetic acid for 10 min, and it was then left to dry in the oven at 80 ℃. The grid was then cut into its individual component squares and was placed into small plastic vials containing 1 mL eluent, 1 mol/L potassium acetate in 70% (v/v) ethanol for 1 h and finally the absorbance of the eluted dye solution was read at 590 nm against the background dye measurements.

Statistical analysis

Data were subjected to 2-tailed paired *t* test and *P* values $\leqslant 0.05$ were considered as statistically significant.

RESULTS

Total phospholipids and total cholesterol in the subcellular fraction of isolated perfused rat livers in the absence and presence of THDC infusion

Total phospholipids and cholesterol in the subcellular fractions from isolated perfused rat livers in the absence and presence of THDC infusion are shown in Figure 1A and B. The total lipids have been expressed as μmol/g of liver due to the differences in liver weight of the animals. No significant differences were found in total phospholipids and cholesterol content of subcellular fractions from control and THDC-infused rat livers (Figure 1A and B).

Total protein and PDE activity in subcellular fractions of liver homogenate

Total protein and PDE activity in subcellular fractions of isolated perfused rat livers in the absence or presence of THDC infusion are depicted in Figure 1C and D. No significant differences were found in total proteins in the subcellular fractions of isolated perfused rat livers in the absence or presence of THDC infusion (Figure 1C). However, PDE activity was significantly higher in fraction P3 Ⅱ from THDC-infused livers (Figure 1D).

Analysis of the subfractions of the microsomal fraction

The microsomal fractions were subjected to self-generating density gradient centrifugation and the fractions were analyzed for total phospholipids, cholesterol, protein and PDE activity. The results are presented in Figure 2. Enzyme activity in THDC-infused liver fractions 1, 2, 8, 9 and 10 was significantly higher when compared to the control values. However, no significant differences were observed in total phospholipids, cholesterol and protein in the subcellular fractions of isolated perfused rat livers in the absence or presence of taurohyodeoxycholic acid infusion.

Biliary PC has a unique fatty acid pattern, 1-palmitoyl 2-linoleyl (16:0-18:2) PC, 1-palmitoyl 2-oleoyl (16:0-18:1) PC, which is distinct from that of membrane PC fatty acid pattern 1-stearoyl 2-arachidonyl (16:0-20:4). No differences in the level of subfractions of PC were found (Table 1). It was not possible to identify peak 1 due to lack of relevant standards (Figure 3).

DISCUSSION

The liver is the site of many important biochemical functions including formation of bile which contains many solutes including phospholipids and cholesterol, both of which are synthesized in the liver and have been implicated in liver and cholestatic disease[29]. Many studies have been performed in which the physical forms of lipids have been isolated in bile; however, very few studies have addressed this problem in the liver. Attempts to isolate vesicles containing biliary type PC and cholesterol have largely been unsuccessful in hepatocytes[5]. However, Gilat and Sömjen[7] and Sömjen *et al*[8] have identified three forms of biliary lipid carriers in bile, namely unilamellar vesicles, stacked lamellae and micelles. The sources of biliary phospholipids may be numerous: *de novo* synthesis, microsomes, Golgi, bile canalicular membrane and preformed hepatic and extrahepatic pool[30]. The extrahepatic pool may contribute about 40% of the biliary phospho-

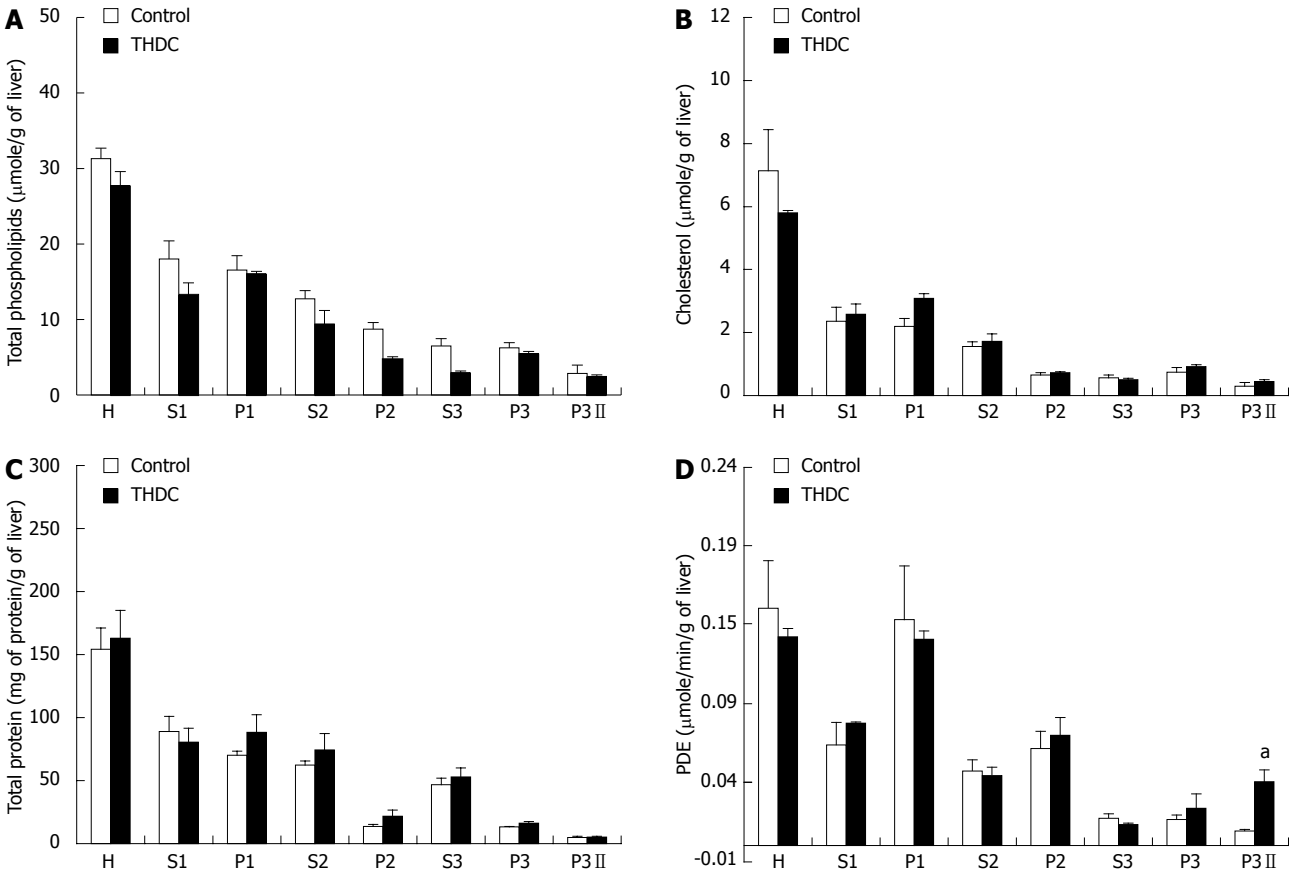

Figure 1 Total phospholipids, cholesterol, protein and phosphodiesterase I activity in subcellular fractions of liver homogenate. A: Phospholipids; B: Cholesterol; C: Protein; D: Phosphodiesterase I activity (PDE). THDC: Taurohyodeoxycholic acid. Symbols are homogenate (H), supernatant 1 (S1), pellet 1 (P1), supernatant 2 (S2), pellet 2 (P2), supernatant 3 (S3), pellet 3 (P3) and pellet 3 (P3 II) diluted with sucrose and KCl. Results are presented as mean ± SE (n = 6). Significant differences from controls were assessed by student's t-test and are indicated by ($^{a}P < 0.05$ *vs* control group).

lipids secreted in the basal state in rats and is associated with high density lipoprotein[31], also bile acid activates a specific cytosolic PC transfer protein in the hepatocytes which then transfers PC to the canalicular membrane. It has also been reported that a PC transmembrane translocator (*flippase*) exists in the canalicular membrane and may be involved in the membrane translocation of specific PC to the biliary side of the canalicular membrane[32]. Most of the cholesterol secreted in bile is derived from circulating plasma lipoprotein, mainly low-density lipoprotein, high-density lipoprotein and chylomicron remnants. Cholesterol is transported probably in vesicles and binds to protein such as sterol carrier protein 2 present in the hepatocytes and, under physiological conditions, biliary bile acid secretion is the driving force behind the secretion of phospholipid and cholesterol in bile[33,34].

THDC is a hydrophilic bile acid, causing more secretion of biliary PC compared to tauroursodeoxycholic acid and taurocholic acid, whereas this bile acid does not significantly increase biliary secretion of cholesterol and protein when compared to the control[20]. It was thought that increased biliary PC carrier vesicles would be present in the hepatocytes in this experiment. This concept was initiated by the study of Angelico *et al*[20], who observed by electron microscopy that increased recruitment of vesicles and lamellar bodies around and within bile canaliculi in the liver occurred with THDC infusion. It is possible that mechanisms at a molecular level include stimulation by THDC of the PC transfer protein and/or of the phospholipid translocator involved in the transmembrane canalicular transport of phospholipids. It has also been reported from physical-chemical and imaging studies that bile salts stimulate the biliary secretion of unilamellar vesicles from the external hemileaflet of the canalicular membrane[31].

However, no significant differences were observed between control and THDC-infused rat liver subfractions in total phospholipids and cholesterol (Figure 1A and B). There was no significant difference in total proteins in the subcellular fractions of isolated perfused rat livers in the absence of THDC infusion (Figure 1C). However, PDE activity in the subcellular fraction P3II was significantly higher than in the corresponding fraction from the control experiment. Within the liver, PDE is a membrane-bound enzyme and would be expected to be associated with vesicles, hence this enzyme was assayed and results may indicate that there is more membrane material in this fraction. This is in contrast to the results reported by Lanzarotto *et al*[35] who observed that chronic administration of THDC in humans with intact

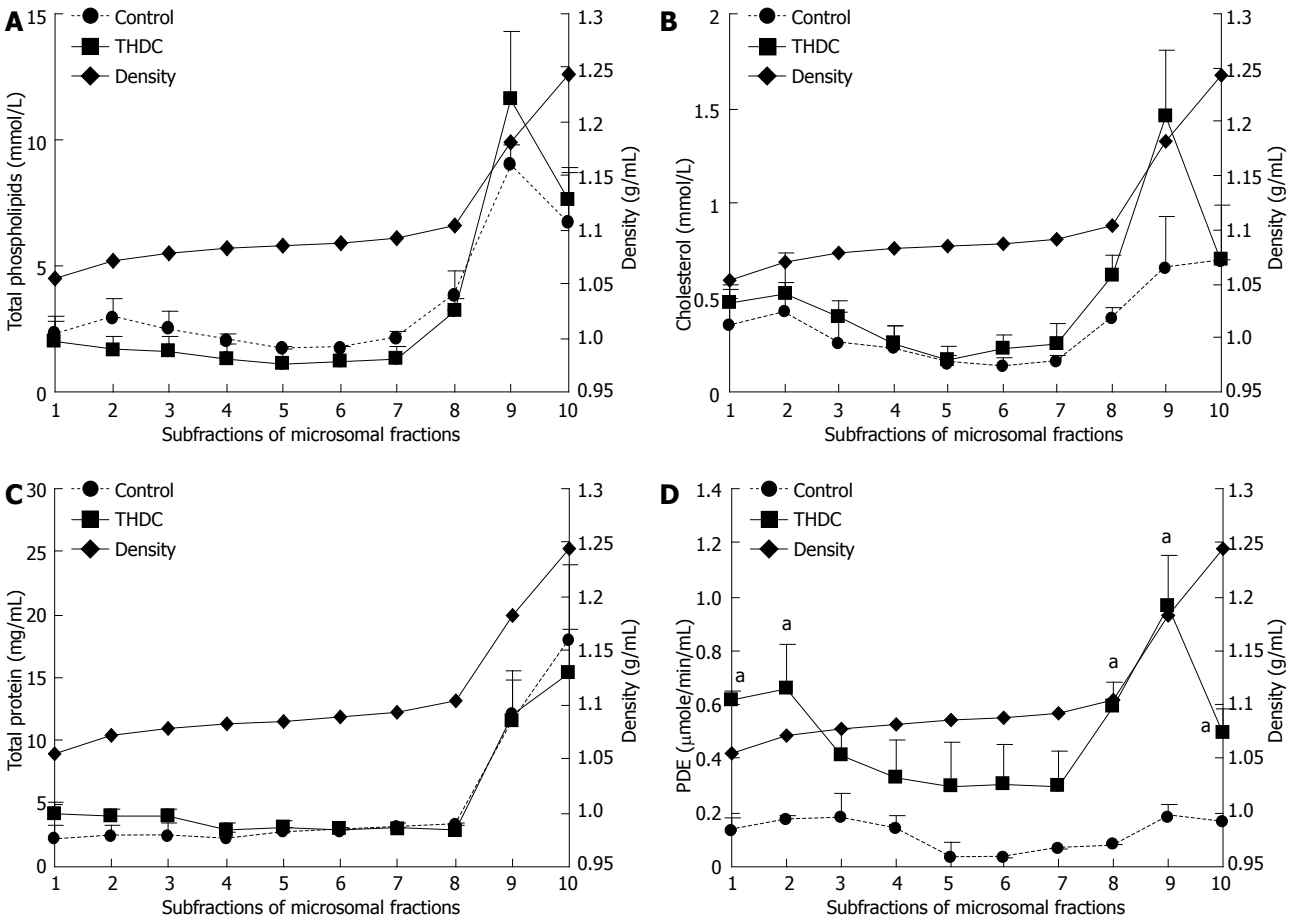

Figure 2 Analysis of the subfractions of the microsomal fraction. A: Phospholipids; B: Cholesterol; C: Protein; D: Phosphodiesterase I activity (PDE). Livers were removed and the microsomal fraction was prepared and subjected to centrifugation and sub-fractions obtained. The sub-fractions were analyzed for total phospholipids, total cholesterol, protein and PDE activity. Results are presented as mean ± SE (n = 6). THDC: Taurohyodeoxycholic acid. Significant differences from controls were assesses by student's t test and indicated by $^{a}P < 0.05$ *vs* control group.

Table 1 High-performance liquid chromatography analysis of the fatty acid composition of phosphatidylcholine

Fractions	PC (nmol)	C16:0 C20:4	C16:0 C18:2	C16:0 C18:1
Control perfused fed rat livers				
1	0.171 ± 0.001	41.147% ± 3.02%	11.048% ± 0.8%	8.327% ± 0.92%
2	0.223 ± 0.003	42.219% ± 2.8%	10.223% ± 0.7%	6.49% ± 0.5%
8	0.214 ± 0.007	41.545% ± 3.09%	11.281% ± 0.8%	6.436% ± 0.9%
9	1.02 ± 0.05	43.59% ± 1.5%	8.68% ± 1%	6.61% ± 0.73%
10	1.34 ± 1.1	44.64% ± 0.27%	8.19% ± 0.64%	6.84% ± 0.33%
THDC-perfused rat livers				
1	0.193 ± 0.001	44.424% ± 3.29%	7.864% ± 0.92%	10.239% ± 1.52%
2	0.208 ± 0.004	44.796% ± 2.09%	8.177% ± 0.8%	8.507% ± 0.93%
8	0.444 ± 0.008	43.735% ± 0.98%	9.373% ± 1.76%	8.298% ± 1.35%
9	1.47 ± 0.16	44.94% ± 1.2%	8.07% ± 0.3%	8.23% ± 0.95%
10	1.17 ± 0.4	43.94% ± 2.8%	7.94% ± 0.52%	7.74% ± 1.7%

Phosphatidylcholine was isolated and separated by thin layer chromatography and then subjected to high-performance liquid chromatography (HPLC) for the sub-fractionation of the fatty acid composition as described in the methods section for control perfused livers and in livers perfused with taurohyodeoxycholic acid (THDC). Results are presented as mean ± SE (n = 6). No significant differences were found between the fatty acid composition of livers perfused with THDC when compared to the control; PC: Phosphatidylcholine.

enterohepatic circulation has little effect on biliary lipid composition and secretion. In contrast, Sinhal *et al*[36] and Cohen *et al*[37] showed that feeding THDC to hamsters and prairie dogs increased hepatic HMG-CoA reductase activity and thus an increase in vesicles carrying cholesterol. It is speculated that a similar increase in the activity of PDE is caused in this experiment by THDC.

Analysis of the microsomal fraction gave an interesting profile of total phospholipids, cholesterol, protein and PDE, as presented in Figure 2. The results indicate that there may be two different populations of the parameters measured. The first population isolated from

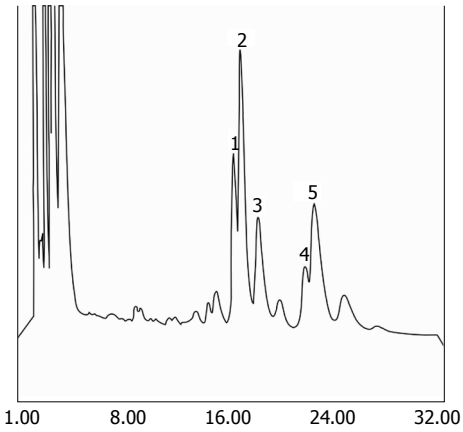

Figure 3 A typical high-performance liquid chromatography chromatogram of a liver homogenate. Phospholipids were separated by thin layer chromatography (TLC) and the phosphatidylcholine band was scraped off the TLC plate and extracted with 2 × 1 mL methanol [high-performance liquid chromatography (HPLC) grade]. After determination of the phospholipid, 20 nmol was injected onto HPLC for the analysis of the fatty acid composition of the phosphatidylcholine as described in the methods section. Results are presented as mean ± SE (n = 6). A typical chromatogram is presented. Peak 1: Not identified; peak 2: 1-palmitoyl 2-arachidonyl (16:0-20:4) phosphatidylcholine; peak 3: 1-palmitoyl 2-linoleyl (16:0-18:2) phosphatidylcholine; peak 4: 1-palmitoyl 2-oleoyl (16:0-18:1) phosphatidylcholine; peak 5: 1-stearol 2-arachidonyl (18:0-20:4) phosphatidylcholine.

fractions 1-2 (ρ = 1.05-1.07 g/mL) had relatively small amounts of cholesterol and PDE activity, whereas the population isolated from fractions 8-10 (ρ = 1.09-1.23 g/mL) had higher concentrations of phospholipids, cholesterol, protein and PDE activity (Figure 2A-D). Some putative vesicles may be present in fractions 8-10 (ρ = 1.09-1.23 g/mL) since these had more total phospholipids, cholesterol, protein and PDE activity. Enzyme activity in THDC-infused liver microsomal sub-fractions (ρ = 1.05-1.07 g/mL and ρ = 1.95-1.23 g/mL) was significantly higher than that observed in control values (Figure 2D). No significant difference was found in PC molecular species in the C16:0-C18:2, C16:0-C18:1 between control and THDC-infused liver subfractions.

In the experiments reported in this study, the isolation of biliary type vesicles was achieved by using the novel gradient medium, Iodixanol, which is a nonionic medium that has an advantage over sucrose in that it rapidly forms self-generated gradients in vertical or near-vertical rotors[38]. Increased lipid transfer vesicles might be present in the microsomal fraction[39,40]; however, subfractions of microsomal fraction on density gradient with Iodixanol failed to identify biliary transfer vesicles.

Crawford *et al*[32,41] reported that vesicles are secreted from the outer leaflet of the canalicular membrane by ABCB4 transporter and subsequently, bile salt/phospholipid micelles in bile extract cholesterol from these vesicles. Vesicular secretion is compatible with the function of ABCG5/ABCG8, and several studies[7,32,42,43] have suggested that vesicular secretion of cholesterol is one of the mechanisms by which sterols appear in bile.

According to the results of this study no significant changes in biliary lipid-carrying vesicles were observed; however, a significantly different profile of PDE was seen. However, the observation of increased vesicle accumulation during bile salt secretion by electron microscopy[44] and inhibition of the vesicle transport by colchicine, vinblastine and valproate still require explanation[45]. The changing of lipid content in any subcellular compartment might be prevented by analysis of the whole liver. However, the subcellular fraction of the livers which was also an initial fraction resulted in no significant difference between control and THDC-perfused rat livers (Figure 1A and B). Some putative biliary lipid transfer vesicles may exist but techniques used in this study have failed to identify them. The identification and regulation of biliary lipid-carrying vesicles may lead to an improvement in the treatment of hepatic disorders.

In conclusion, the present study failed to identify an increase in biliary lipid-carrying vesicles in THDC-infused rat livers probably due to the limitation of the techniques. However, PDE activity was significantly increased in the microsomal sub-fractions isolated from THDC-infused livers when compared to control values and needs further investigation.

COMMENTS

Background

Bile contains many constituents, including cholesterol and phospholipids, and these are reported to be transported from the hepatocytes to the bile canaliculus in vesicles. An increase in biliary lipid secretion can have implications for hepatic disorders such as cholesterol gallstone formation. Hence, the isolation of biliary lipid-carrying vesicles may lead to a better understanding of such hepatic disorders.

Research frontiers

Taurohyodeoxycholic acid (THDC) is reported to increase biliary lipid-carrying vesicles (mainly phosphatidylcholine) and ultracentrifugation techniques are now available which can be used to separate vesicle type material relatively quickly.

Innovations and breakthroughs

Biliary phosphatidylcholine has a unique fatty acid composition compared to hepatic phosphatidylcholine and can be identified by high-performance liquid chromatography (HPLC). Since THDC induces an increase in biliary phosphatidylcholine it was thought that increased biliary phosphatidylcholine carrier vesicles would be present in the hepatocytes and could be separated by ultracentrifugation. Electron microscopy has confirmed the presence of increased vesicles and lamellar bodies around and within the bile canaliculus after THDC infusion.

Applications

Although the present study failed to identify an increase in biliary lipid-carrying vesicles in THDC-infused livers, probably due to the limitation of the techniques employed, vesicles enriched in phosphodiesterase I (PDE) activity were present in THDC livers compared to controls.

Terminology

THDC is a natural 6α-hydroxylated bile acid displaying hydrophilic properties and causes more secretion of phosphatidylcholine into bile compared to other bile acids. The microsomal fraction was obtained and subjected to further ultracentrifugation using a novel gradient centrifugation technique. The phosphatidylcholine was separated and subjected to HPLC fractionation since biliary phosphatidylcholine has a unique fatty acid composition.

Peer review

In this study the authors have isolated rat livers and have subjected them to THDC infusion, which is reported to increase biliary phospholipid secretion. The livers were then homogenized and the microsomal fraction was subjected to ultracentrifugation by using a novel gradient technique in order to isolate putative vesicles destined for biliary secretion. The results show that the density

gradient fraction envisaged to contain the putative vesicle population isolated from THDC-perfused livers had relatively small amounts of phospholipids and protein when compared to the relevant control fractions. However, the vesicles isolated from the THDC-perfused livers displayed an increase in cholesterol and PDE activity.

REFERENCES

1 **Dikkers A**, Tietge UJ. Biliary cholesterol secretion: more than a simple ABC. *World J Gastroenterol* 2010; **16**: 5936-5945 [PMID: 21157969 DOI: 10.3748/wjg.v16.i47.5936]
2 **Zanlungo S**, Nervi F. The molecular and metabolic basis of biliary cholesterol secretion and gallstone disease. *Front Biosci* 2003; **8**: s1166-s1174 [PMID: 12957839]
3 **Booker ML**, Scott TE, La Morte WW. Effect of dietary cholesterol on phosphatidylcholines and phosphatidylethanolamines in bile and gallbladder mucosa in the prairie dog. *Gastroenterology* 1989; **97**: 1261-1267 [PMID: 2507386]
4 **White DA**. In: Ansell GB, Hawthorne JN, Dawson RMC, editors. Form and Function of Phospholipids. New York: Elsevier Scientific, 1973: 441-482
5 **Verkade HJ**, Vonk RJ, Kuipers F. New insights into the mechanism of bile acid-induced biliary lipid secretion. *Hepatology* 1995; **21**: 1174-1189 [PMID: 7705793]
6 **Nervi F**, Marinović I, Rigotti A, Ulloa N. Regulation of biliary cholesterol secretion. Functional relationship between the canalicular and sinusoidal cholesterol secretory pathways in the rat. *J Clin Invest* 1988; **82**: 1818-1825 [PMID: 3198756 DOI: 10.1172/JCI113797]
7 **Gilat T**, Sömjen GJ. Phospholipid vesicles and other cholesterol carriers in bile. *Biochim Biophys Acta* 1996; **1286**: 95-115 [PMID: 8652613]
8 **Sömjen GJ**, Marikovsky Y, Wachtel E, Harvey PR, Rosenberg R, Strasberg SM, Gilat T. Phospholipid lamellae are cholesterol carriers in human bile. *Biochim Biophys Acta* 1990; **1042**: 28-35 [PMID: 2297522 DOI: 10.1016/0005-2760(90)90052-Y]
9 **Crawford JM**. Intracellular traffic and plasma membrane secretion of small organic solutes involved in hepatocellular bile formation. *Comp Biochem Physiol* 1996; **115B**: 341-354
10 **Graf GA**, Yu L, Li WP, Gerard R, Tuma PL, Cohen JC, Hobbs HH. ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. *J Biol Chem* 2003; **278**: 48275-48282 [PMID: 14504269 DOI: 10.1074/jbcM310223200]
11 **Kamisako T**, Ogawa H. Regulation of biliary cholesterol secretion is associated with abcg5 and abcg8 expressions in the rats: effects of diosgenin and ethinyl estradiol. *Hepatol Res* 2003; **26**: 348-352 [PMID: 12963437 DOI: 10.1016/S1386-6346(03)00153-0]
12 **Hişmioğullari AA**, Bozdayi AM, Rahman K. Biliary lipid secretion. *Turk J Gastroenterol* 2007; **18**: 65-70 [PMID: 17602352]
13 **Bloks VW**, Bakker-Van Waarde WM, Verkade HJ, Kema IP, Wolters H, Vink E, Groen AK, Kuipers F. Down-regulation of hepatic and intestinal Abcg5 and Abcg8 expression associated with altered sterol fluxes in rats with streptozotocin-induced diabetes. *Diabetologia* 2004; **47**: 104-112 [PMID: 14618236 DOI: 10.1007/s00125-003-1261-y]
14 **Kamisako T**, Ogawa H. Effect of obstructive jaundice on the regulation of hepatic cholesterol metabolism in the rat. Disappearance of abcg5 and abcg8 mRNA after bile duct ligation. *Hepatol Res* 2003; **25**: 99-104 [PMID: 12644045 DOI: 10.1016/S1386-6346(02)00248-6]
15 **Kosters A**, Frijters RJ, Kunne C, Vink E, Schneiders MS, Schaap FG, Nibbering CP, Patel SB, Groen AK. Diosgenin-induced biliary cholesterol secretion in mice requires Abcg8. *Hepatology* 2005; **41**: 141-150 [PMID: 15619238 DOI: 10.1002/hep.20540]
16 **Kosters A**, Kunne C, Looije N, Patel SB, Oude Elferink RP, Groen AK. The mechanism of ABCG5/ABCG8 in biliary cholesterol secretion in mice. *J Lipid Res* 2006; **47**: 1959-1966 [PMID: 16741293 DOI: 10.1194/jlr.M500511-JLR200]
17 **Yu L**, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, Hobbs HH. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest* 2002; **110**: 671-680 [PMID: 12208868 DOI: 10.1172/JCI16001]
18 **Yu L**, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci USA* 2002; **99**: 16237-16242 [PMID: 12444248 DOI: 10.1073/pnas252582399]
19 **Hazard SE**, Patel SB. Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. *Pflugers Arch* 2007; **453**: 745-752 [PMID: 16440216 DOI: 10.1007/s00424-005-0040-7]
20 **Angelico M**, Baiocchi L, Nistri A, Franchitto A, Della Guardia P, Gaudio E. Effect of taurohyodeoxycholic acid, a hydrophilic bile salt, on bile salt and biliary lipid secretion in the rat. *Dig Dis Sci* 1994; **39**: 2389-2397 [PMID: 7956608]
21 **Roda A**, Piazza F, Baraldini M, Speroni E, Guerra MC, Cerré C, Cantelli Forti G. Taurohyodeoxycholic acid protects against taurochenodeoxycholic acid-induced cholestasis in the rat. *Hepatology* 1998; **27**: 520-525 [PMID: 9462652]
22 **Rahman K**, Coleman R. Output of lysosomal contents and cholesterol into bile can be stimulated by taurodehydrocholate. *Biochem J* 1987; **245**: 289-292 [PMID: 3663154]
23 **Ford TC**, Graham JM. An introduction to centrifugation. Oxford: BIOS, Scientific Publishers, 1991 [DOI: 10.1016/0307-4412(91)90066-H]
24 **Zak B**, Dickenman RC, White EG, Burnett H, Cherney PJ. Rapid estimation of free and total cholesterol. *Am J Clin Pathol* 1954; **24**: 1307-1315 [PMID: 13207063]
25 **Bligh EG**, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; **37**: 911-917 [PMID: 13671378]
26 **Bartlett GR**. Phosphorus assay in column chromatography. *J Biol Chem* 1959; **234**: 466-468 [PMID: 13641241]
27 **Trams EG**, Lauter CJ. On the sidedness of plasma membrane enzymes. *Biochim Biophys Acta* 1974; **345**: 180-197 [PMID: 4366461 DOI: 10.1016/0005-2736(74)90257-0]
28 **Winterbourne DJ**. Chemical Assays for Proteins. In: Graham JM, Higgins JA, editors. Biomembrane Protocols. Totowa: Humana Press Inc, 1993 [DOI: 10.1385/0-89603-236-1:197]
29 **Abeysuriya V**, Deen KI, Navarathne NM. Biliary microlithiasis, sludge, crystals, microcrystallization, and usefulness of assessment of nucleation time. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 248-253 [PMID: 20525550]
30 **Yousef IM**, Bloxam DL, Phillips MJ, Fisher MM. Liver cell plasma membrane lipids and the origin of biliary phospholipid. *Can J Biochem* 1975; **53**: 989-997 [PMID: 1182591]
31 **Chanussot F**, Lafont H, Hauton J, Tuchweber B, Yousef I. Studies on the origin of biliary phospholipid. Effect of dehydrocholic acid and cholic acid infusions on hepatic and biliary phospholipids. *Biochem J* 1990; **270**: 691-695 [PMID: 2241901]
32 **Crawford AR**, Smith AJ, Hatch VC, Oude Elferink RP, Borst P, Crawford JM. Hepatic secretion of phospholipid vesicles in the mouse critically depends on mdr2 or MDR3 P-glycoprotein expression. Visualization by electron microscopy. *J Clin Invest* 1997; **100**: 2562-2567 [PMID: 9366571 DOI: 10.1172/JCI119799]
33 **Barnwell SG**, Tuchweber B, Yousef IM. Biliary lipid secretion in the rat during infusion of increasing doses of unconjugated bile acids. *Biochim Biophys Acta* 1987; **922**: 221-233 [PMID: 3676344 DOI: 10.1016/0005-2760(87)90158-5]
34 **Venneman NG**, van Erpecum KJ. Pathogenesis of gallstones. *Gastroenterol Clin North Am* 2010; **39**: 171-83, vii [PMID: 20478481 DOI: 10.1016/j.gtc.2010.02.010]
35 **Lanzarotto F**, Sosta S, Lanzini A. Effect of chronic administration of tauro-hyodeoxycholic acid on biliary bile acid

composition and on biliary lipid secretion in humans. *Scand J Gastroenterol* 2001; **36**: 981-986 [PMID: 11521991]
36 **Singhal AK**, Cohen BI, Finver-Sadowsky J, McSherry CK, Mosbach EH. Role of hydrophilic bile acids and of sterols on cholelithiasis in the hamster. *J Lipid Res* 1984; **25**: 564-570 [PMID: 6547738]
37 **Cohen BI**, Mosbach EH, McSherry CK, Stenger RJ, Kuroki S, Rzigalinski B. Gallstone prevention in prairie dogs: comparison of chow vs. semisynthetic diets. *Hepatology* 1986; **6**: 874-880 [PMID: 3758942]
38 **Billington D**, Maltby PJ, Jackson AP, Graham JM. Dissection of hepatic receptor-mediated endocytic pathways using self-generated gradients of iodixanol (Optiprep). *Anal Biochem* 1998; **258**: 251-258 [PMID: 9570838 DOI: 10.1006/abio.1998.2561]
39 **Yamaguchi A**, Tazuma S, Ochi H, Chayama K. Choleretic action of diosgenin is based upon the increases in canalicular membrane fluidity and transporter activity mediating bile acid independent bile flow. *Hepatol Res* 2003; **25**: 287-295 [PMID: 12697250 DOI: 10.1016/S1386-6346(02)00268-1]
40 **Amigo L**, Mendoza H, Zanlungo S, Miquel JF, Rigotti A, González S, Nervi F. Enrichment of canalicular membrane with cholesterol and sphingomyelin prevents bile salt-induced hepatic damage. *J Lipid Res* 1999; **40**: 533-542 [PMID: 10064742]
41 **Crawford JM**, Möckel GM, Crawford AR, Hagen SJ, Hatch VC, Barnes S, Godleski JJ, Carey MC. Imaging biliary lipid secretion in the rat: ultrastructural evidence for vesiculation of the hepatocyte canalicular membrane. *J Lipid Res* 1995; **36**: 2147-2163 [PMID: 8576641]
42 **Sömjen GJ**, Marikovsky Y, Lelkes P, Gilat T. Cholesterol-phospholipid vesicles in human bile: an ultrastructural study. *Biochim Biophys Acta* 1986; **879**: 14-21 [PMID: 3768382 DOI: 10.1016/0005-2760(86)90260-2]
43 **Poupon R**, Poupon R, Dumont M, Erlinger S. Hepatic storage and biliary transport maximum of taurocholate and taurochenodeoxycholate in the dog. *Eur J Clin Invest* 1976; **6**: 431-437 [PMID: 1001345]
44 **Barnwell SG**, Lowe PJ, Coleman R. The effects of colchicine on secretion into bile of bile salts, phospholipids, cholesterol and plasma membrane enzymes: bile salts are secreted unaccompanied by phospholipids and cholesterol. *Biochem J* 1984; **220**: 723-731 [PMID: 6466298]
45 **Marzolo MP**, Rigotti A, Nervi F. Secretion of biliary lipids from the hepatocyte. *Hepatology* 1990; **12**: 134S-141S; discussion 141S-142S [PMID: 2210641]

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BRIEF ARTICLE

Nilotinib-mediated mucosal healing in a rat model of colitis

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Abstract

AIM: To investigate the effects of nilotinib in a rat model of trinitrobenzene sulfonic acid (TNBS)-induced colitis.

METHODS: Twenty-one Wistar albino female rats obtained from Dokuz Eylul University Department of Laboratory Animal Science were categorized into a control (n = 7), TNBS (n = 7) and nilotinib group (n = 7). Saline was administered orally for 14 d to the control and the TNBS group. The TNBS group received rectal TNBS on the first day while saline was administered to the control group. The nilotinib group received 20 mg/kg nilotinib for 14 d in 2 divided doses, starting the same day as TNBS administration. For 14 d, the rats were fed a standard diet, and their weights were recorded daily. After sacrifice, colon tissue samples from each group were scored for macroscopic and microscopic pathology. Apoptotic indices were determined by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling method. Platelet-derived growth factor receptor (PDGFR) alpha and beta levels were assessed through immunohistochemistry staining scores and compared among the groups. Tissue and serum tumor necrosis factor (TNF) alpha levels were determined by enzyme-linked immunosorbent assay.

RESULTS: Between days 1 and 14, the nilotinib group rats lost significantly less weight than the TNBS group rats (-0.7 g *vs* -14.0 g, P = 0.047). The difference in weight between the control and nilotinib groups was also statistically significant (+8.3 g *vs* -0.7 g, P = 0.031). From day 7 to day 14, the weight differences of the control group *vs* the TNBS group, the TNBS group *vs* the nilotinib group, and the control group *vs* the nilotinib group were all statistically significant (+8.0 g *vs* -11.1 g, P = 0.007; -11.1 g *vs* +2.9 g, P = 0.015; +8.0 g *vs* +2.9 g, P = 0.042, respectively). Macroscopic and microscopic scores were significantly lower in the nilotinib group than in the TNBS group (0.00 ± 0.00 *vs* 1.43 ± 0.65, P = 0.009; 2.86 ± 0.55 *vs* 7.71 ± 1.48, P = 0.030, respectively). However, these scores were similar between the nilotinib and control groups. While no significant difference for the nilotinib *vs* control groups could be determined for PDGFR alpha and beta scores, PDGFR alpha and beta scores were lower in the nilotinib group than in the TNBS group. Furthermore, the TNF alpha levels in the serum, tissue and apoptosis scores were similar between the nilotinib and TNBS groups.

CONCLUSION: Nilotinib prevents weight loss, facilitates mucosal healing by improving the pathological scores without introducing variation into the apoptotic scores or TNF alpha levels.

Key words: Inflammatory bowel disease; Platelet-derived growth factor receptor; Tumor necrosis factor alpha; Tyrosine kinase inhibitor; Mucosal healing

Core tip: Unresponsiveness to medical treatment in refractory inflammatory bowel disease (IBD) still poses a therapeutic challenge. To detect an alternative treatment option, we selected nilotinib based on the fact that tyrosine kinases inhibitors affect several key components in the pathogenesis of IBD, including tumor necrosis factor (TNF) alpha, platelet-derived growth factor receptor (PDGFR), and apoptosis. In a trinitrobenzene sulfonic acid-induced colitis rat model, we concluded that nilotinib has a significant effect on weight loss and on macroscopic and microscopic pathological scores, leading to significant mucosal healing. Although nilotinib caused a decrease in the PDGFR alpha and PDGFR beta levels, it did not have a significant effect on the apoptotic scores or TNF alpha levels.

Ataca P, Soyturk M, Karaman M, Unlu M, Sagol O, Dervis Hakim G, Yilmaz O. Nilotinib-mediated mucosal healing in a rat model of colitis. *World J Gastroenterol* 2013; 19(37): 6237-6244 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6237.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6237

INTRODUCTION

Chronic intestinal inflammation is characterized by the pathological responses of the adaptive and innate immune systems. These responses are central to the pathological mechanisms that lead to inflammatory bowel disease (IBD)[1]. Genetic and environmental factors, infectious agents, the structure of the enteric flora, and immune system dysfunctions are key elements in the pathogenesis of IBD, and thus, these are targets for many drugs developed to treat IBD[2,3]. However, unresponsiveness to medical treatment in IBD still poses a therapeutic challenge. Previous studies examining the therapeutic effectiveness of selecting drugs in patients with ulcerative colitis (UC) reported the rates of remission to be 47%-81% with rectal 5-aminosalicylic acid (5-ASA), 9%-30% with oral 5-ASA, and 42%-82% with thiopurines[4-6].

Monoclonal tumor necrosis factor (TNF) alpha inhibitors are currently the treatment of choice, especially in severe and resistant cases of IBD. However, decreased responses or resistance to the TNF alpha inhibitor infliximab have been reported. Previous studies have reported an average clinical remission rate at week 8 of 33% (range, 27.5%-38.8%) with the use of infliximab in IBD patients[7]. Clinical remission was maintained in 33% (range, 25.6%-36.9%) of patients treated with infliximab at week 30[7]. In a randomized, placebo-controlled 52-wk study examining the effectiveness of adalimumab, another anti-TNF agent, the IBD remission rate was significantly higher than the placebo, regardless of treatment with steroids (13.3% and 5.7%, respectively; $P = 0.035$)[8].

Mucosal healing has emerged as a key therapeutic objective in the treatment of IBD and is able to predict sustained clinical remission and resection-free survival in patients. Mucosal healing is achieved in approximately 30% of IBD patients receiving corticosteroid therapy and in as many as 60% of IBD patients receiving anti-TNF therapies[9-11]. Approximately 20% of IBD patients, however, do not respond to anti-TNF therapy and require surgical intervention[12]. These findings emphasize the importance of discovering new medical treatment options for IBD because the currently available treatments are insufficient for a substantial number of patients.

Tyrosine kinases (TKs) are enzymes that play a role in normal cell function, metabolism, growth, differentiation, and apoptosis. TK inhibitors are drugs that block the action of these enzymes. Although they are typically used as anticancer drugs, they have recently been considered for use in noncancer proliferative diseases and for inflammatory conditions. Imatinib, the best-known member of this class of drugs, is specific for TK receptor sites and suppresses the Abelson proto-oncogene (ABL), the c-kit proto-oncogene, platelet-derived growth factor receptor (PDGFR), macrophage colony-stimulating factor receptor, TNF alpha, and inducible nitric oxide synthase[13]. Nilotinib is a more potent inhibitor of TKs than imatinib. In studies involving patients with lung fibrosis, nilotinib has been shown to reduce interleukin (IL)-6, IL-1 beta, TNF alpha, tumor growth factor beta 1, and PDGFR beta levels more significantly than imatinib and had a potent antifibrotic effect[14].

In the literature, there are a few reports suggesting that TK inhibitors may be effective in IBD. In a case report by Magro *et al*[15], a patient diagnosed with Crohn's disease (CD) and chronic myeloid leukemia (CML) remained in remission for 3 years on imatinib therapy alone, without the use of mesalamine or steroids. Cuzzocrea *et al*[16] demonstrated that the development of colitis in dinitrobenzene sulfonic acid (DNBS)-induced colitis animal models was reduced by the TK inhibitor tyrphostin AG126.

The present study was planned based on the demonstrated success of nilotinib in previous studies and on the fact that TK inhibitors affect several key components in the pathogenesis of IBD, including TNF alpha, PDGFR, and nitric oxide (NO) synthesis. For this purpose, we evaluated the efficacy of nilotinib on weight, macroscopic and microscopic pathological scores, TNF alpha levels, PDGFR levels, and the apoptotic index in a rat model of trinitrobenzene sulfonic acid (TNBS)-induced colitis. This study is the first to evaluate the efficacy of nilotinib in a rat colitis model.

MATERIALS AND METHODS

Experimental design

Approval was obtained from the animal ethics council of Dokuz Eylul University Medical Faculty (DEUTF). The

DEUTF Hospital Experimental Research Laboratory provided 21 female Wistar albino rats weighing 200-250 g (mean weight: 209.43 ± 8.92 g) for use in this study.

The rats were maintained in a room at a temperature of 23 ± 2 ℃ under a 12-h light/dark cycle at the DEUTF Experimental Animal Laboratory. Before and during the study, they were fed a standard diet, and their weights were monitored daily. The animals were also allowed water *ad libitum*.

The rats were divided into 3 groups, each consisting of 7 rats: the control group, TNBS group and nilotinib group. After 24 h of fasting, 0.25 mL of the physiological serum was intracolonically administered to the control group rats through a cannula inserted 8 cm proximal to the anus, using a rectally inserted flexible polypropylene catheter. To induce colitis, the rats in the other 2 groups received an intracolonic solution treated with 0.5 mL of 100 mg/mL TNBS (Sigma, Germany) dissolved in 30% ethanol and administered through a cannula. Before catheter insertion, short-term sedation was provided through ether anesthesia. Neither group of rats treated with TNBS encounter any instance of perforation or death due to colonic ulceration. The TNBS and control groups received a saline placebo for 14 d through an orogastric tube. Nilotinib 20 mg/kg/d (Novartis Pharma AG, Basel, Switzerland) was administered in 2 divided doses to the nilotinib group for 14 d through an orogastric tube, beginning on the same day as TNBS administration.

Blood and tissue samples for pathological examination were obtained from all of the rats under ether anesthesia at the end of the 14-d period. All of the animals were then sacrificed by decapitation. The abdominal cavities were opened by a midline incision, and the entire length of the large intestines was dissected from the distal ileum to the rectum. After washing with saline, the large intestinal tissues were fixed with buffered formalin.

Pathological examination

A pathologist blinded to the group identity of the intestinal samples performed pathological evaluations of all of the tissue samples. Each intestinal column was opened longitudinally, according to the method reported by Vilaseca *et al*[17], and macroscopic scoring was performed. Tissue sections of the gross ulcerative lesions and surrounding normal mucosa were then stained with hematoxylin-eosin (HE). The pathologist then performed microscopic scoring according to the method reported by Dieleman *et al*[18].

Apoptosis

The pathologist stained all tissue samples using the TUNEL method. Mucosal crypts and apoptotic cells were counted along the surface epithelium under a microscope (Olympus DX51) at a magnification of × 400. Using the TUNEL technique, all of the cut sections were preserved with lysine for 3 nights at 37 ℃ and then for 1 night at 60 ℃ in an incubator. Thereafter, deparaffinization was performed with 3 cycles of xylene. The tissue sections were then rehydrated by flushing with a series of alcohol solutions of decreasing degrees (absolute, 96%, 80%, and 70%) and then stored in distilled water for 5 min. Proteinase K (Proteinase K, Invitrogen, Carlsbad, CA, United States) was applied for 10 min at room temperature. The sections were then washed twice with phosphate-buffered solution (PBS) for a period of 2 min each. After drying the cross-sections, 3% H_2O_2 (Merck, Germany) was applied for 5 min, and the sections were then washed with PBS twice for 5 min each. The cross-sectional slices were then dried, and an equilibration buffer (ApopTag Plus peroxidase kit, Millipore, Billerica, MA, United States) was applied for 10 min at room temperature. A total of 55 μL of the enzyme terminal deoxynucleotidyl transferase was then applied to each cross-section. The cross-sections were closed with a coverslip (ApopTag Plus peroxidase kit, Millipore, Billerica, MA, United States) and incubated for 1 h at 37 ℃. Stop/wash buffer (ApopTag Plus peroxidase kit, Millipore, Billerica, MA, United States) was then applied to the sections removed from the incubator for 10 min at room temperature. The sections were then washed with PBS at room temperature 3 times for 1 min each, dried, and incubated with anti-streptavidin-peroxidase (ApopTag Plus peroxidase kit, Millipore, Billerica, MA, United States) at room temperature for 30 min. The sections were then washed with PBS 4 times for 2 min to determine the visibility of the TUNEL reaction before being stained with diaminobenzidine (DAB) (DAB-PLUS kit; Invitrogen, Carlsbad, CA, United States). After washing with distilled water, ground staining was performed using methyl green. After three changes of the searing process with xylene for 20 min, it was closed with Entella.

Tissue homogenization and measurement of tissue serum TNF alpha

The tissue samples obtained from the ileum were introduced into 2 mL microcentrifuge tubes and stored at -80° C until the day of the study. These tissues were then removed and warmed to 4 ℃. Next, 60-80 mg pieces were obtained from these samples and placed into a tube containing 5-mm-diameter stainless steel beads and a phosphate buffer with a 1:7 ratio (pH 7.2). Microcentrifuge tubes were introduced into a pre-chilled TissueLyser LT device and replaced into a TissueLyser (Qiagen-Germany) tissue homogenization device. Next, an enzyme-linked immunosorbent assay (ELISA) was performed on tissue supernatants, and serum was obtained via centrifugation for the identification of TNF alpha in accordance with the manufacturer's recommendations (Invitrogen Rat TNF-alpha, Carlsbad, CA, United States). Finally, the ELISA plates were spectrophotometrically evaluated at 450 nm (Biotech Synergy HT; Winooski, VT, United States).

PDGFR alpha and beta levels

PDGFR alpha and beta levels were assessed through staining scores and compared among the groups by im-

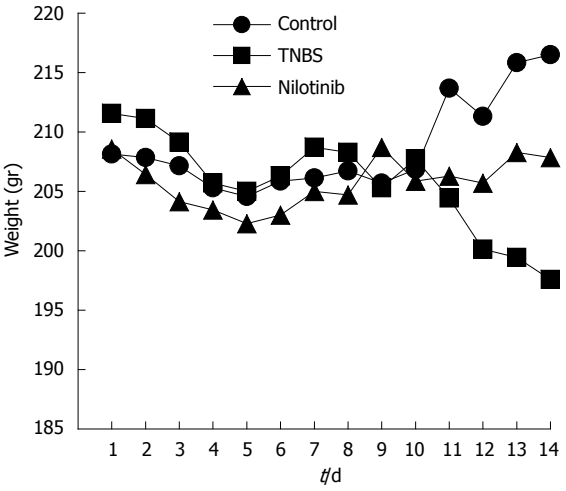

Figure 1 Trends of weight changes among the experimental groups. Control group (circle), Trinitrobenzene sulfonic acid (TNBS) group (square), and nilotinib group (triangle). The TNBS group lost an average weight of 14 g, while the nilotinib group lost 0.7 g in 14 d (P = 0.047). The nilotinib group gained an average weight of 2.9 g between day 7 and day 14, while the TNBS group lost an average weight of 11.1 g (P = 0.015).

munohistochemistry. For immunohistochemical staining, 2-3 micron sections were stored overnight in an incubator at 40 ℃. The following day, the sections were washed with xylene, a descending alcohol series, and distilled water for 20 min. They were then boiled for 20 min in EDTA solution at pH 8. Next, they were stored in DakoFlex peroxidase solution for 5 min and washed again with Tris-buffered saline. A primary antibody was then applied. PDGFR alpha in a 1:100 dilution (NOVUS Biologicals, NBP1-19 423, Littleton, CO, United States) and PDGFR beta in a 1:50 dilution (NOVUS Biologicals, NBP1-19 473; Littleton, CO, United States) were stored for 30 min, washed with Tris buffer, stored in DakoFlex HRP solution for 20 min, washed with Tris buffer again, and stored in DakoFlex DAB for 7 min. The samples were again washed with Tris-buffered saline, kept under tap water for 5 min, stained with Mayer's hematoxylin solution for 10 min, washed with tap water for 1 min, rinsed in an alcohol series, and cleaned with xylene for 5-10 min.

PDGFR alpha and beta positivity was determined according to a devised scoring system. According to this system, a score of 1 was assigned if PDGFR alpha and beta positivity was confirmed in inflammatory cells and in the cells of the lamina propria, stroma, and submucosal endothelium. A score of 2 was assigned if increased expression of PDGFR alpha and beta was confirmed in the lamina propria and submucosa. A score of 3 was assigned if PDGFR alpha and beta positivity was confirmed with widespread staining in the ulcerated areas or in the inflammatory cells, fibroblasts, endothelial cells, submucosa, and mucosa of the surrounding tissue.

Statistical analysis

All statistical procedures were performed using SPSS software (version 15.0). The Kruskal-Wallis test was used for multigroup comparisons, and the Mann-Whitney U test was used to compare the means of 2 groups. A P value less than 0.05 was considered statistically significant.

RESULTS

Bloody diarrhea was observed on day 1 of rectal TNBS administration in all 14 of the rats in the 2 experimental groups; no bloody diarrhea was observed in the control group. In the TNBS and nilotinib groups, the diarrhea was semi-solid on day 5. In the nilotinib group, normal stools were observed after day 7. During rectal saline administration under ether anesthesia in the control group, respiratory arrest developed in 1 rat, which remained stable after CPR. However, the animal's general condition deteriorated over the next few d, and the animal died on day 6 of the experiment. An autopsy was not performed on this rat.

On the first experimental day, the average weights were similar among all of the study groups (P > 0.05), and the average weights were examined daily (Figure 1). The average weight of the control group increased to 8.3 g at the end of 14 d. The TNBS group, however, lost an average of 14 g throughout the study, and the nilotinib group lost an average of 0.7 g. There was a significant difference among the groups with regard to the average weight change throughout the study (P = 0.006). The difference in weight between the control and nilotinib groups was statistically significant (+8.3 and -0.7 g, respectively, P = 0.031). The TNBS group lost significantly more weight than the nilotinib group (-14.0 and -0.7 g, respectively; P = 0.047) and the control group (-14.0 and +8.3 g, respectively, P = 0.008).

Between day 7 and day 14, the weights of the control group increased by an average of 8 g; those of the nilotinib group increased by an average of 2.9 g; and those of the TNBS group decreased by an average of 11.1 g. Comparing the average increase in weights over this time period among all 3 of the groups, there was a significant difference observed (P = 0.004). From day 7 to day 14, the weight differences of the control rats *vs* the TNBS rats, the TNBS rats *vs* the nilotinib rats, and the control rats *vs* the nilotinib rats were statistically significant (+8.0 and -11.1 g, P = 0.007; -11.1 and +2.9 g, P = 0.015; +8.0 and +2.9 g, P = 0.042, respectively).

The mean macroscopic pathological scores of the control and nilotinib groups were 0, while the macroscopic pathological score in the TNBS group was 1.43 ± 0.65. When the distribution of macroscopic scores based on rats was examined, all scores from the control and nilotinib group rats were "0", which is noteworthy. The control and nilotinib groups were similar in terms of macroscopic scores (P > 0.05). Macroscopic scores were significantly lower in the control and nilotinib groups than in the TNBS group (0.00 ± 0.00 and 1.43 ± 0.65, P = 0.014; 0.00 and 1.43 ± 0.65, P = 0.009, respectively) (Figure 2).

The mean microscopic scores in the control, TNBS, and nilotinib groups were 2.0 ± 0.45, 7.71 ± 1.48, and 2.86 ± 0.55, respectively. The mean microscopic scores were significantly lower in the control and nilotinib groups than

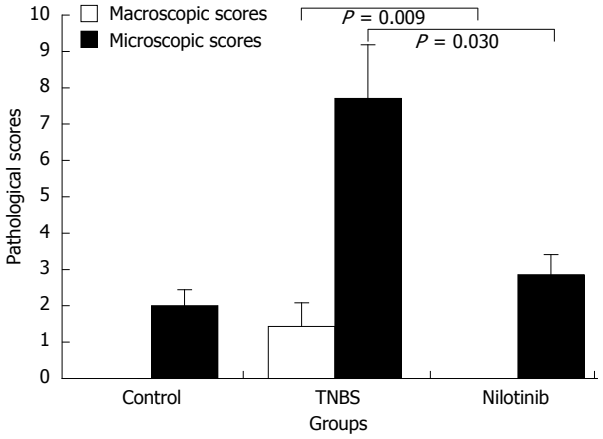

Figure 2 Microscopic and macroscopic pathological scores among the experimental groups. The results are the mean ± SD. Macroscopic and microscopic pathological scores were similar in the control and nilotinib groups, while the scores in the nilotinib group were significantly lower than those in the trinitrobenzene sulfonic acid (TNBS) group (TNBS *vs* nilotinib, $P = 0.009$; TNBS *vs* nilotinib, $P = 0.030$).

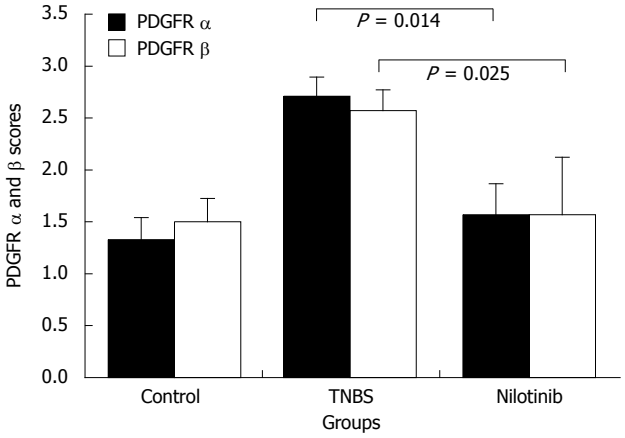

Figure 3 Platelet-derived growth factor receptor alpha and beta scores among the experimental groups. The results are the mean ± SD. Platelet-derived growth factor receptor (PDGFR) alpha and beta scores were similar in the control and nilotinib groups, while the scores in the nilotinib group were significantly lower than those in the trinitrobenzene sulfonic acid (TNBS) group (TNBS *vs* nilotinib, $P = 0.014$; TNBS *vs* nilotinib, $P = 0.025$).

in the TNBS group (2.0 ± 0.45 and 7.71 ± 1.48, $P = 0.034$; 2.86 ± 0.55 and 7.71 ± 1.48, $P = 0.030$, respectively). The control and nilotinib groups were similar in terms of the mean microscopic scores ($P > 0.05$) (Figure 2).

With regard to the PDGFR alpha and beta scoring system, the PDGFR alpha scores in the control, TNBS, and nilotinib groups were 1.33 ± 0.21, 2.71 ± 0.18, and 1.57 ± 0.30, respectively. There was a significant difference among the groups ($P = 0.007$). The PDGFR alpha scores were significantly lower in the control and nilotinib groups than in the TNBS group (1.33 ± 0.21 and 2.71 ± 0.18, $P = 0.004$; 1.57 ± 0.30 and 2.71 ± 0.18, $P = 0.014$, respectively). The control and nilotinib groups were similar in terms of the PDGFR alpha scores ($P > 0.05$) (Figure 3).

The mean PDGFR beta scores in the control, TNBS, and nilotinib groups were 1.50 ± 0.22, 2.57 ± 0.20, and 1.57 ± 0.30, respectively. There was a statistically significant difference among all of the groups in terms of the mean PDGFR beta scores ($P = 0.020$). The PDGFR beta scores were significantly lower in the control and nilotinib groups than in the TNBS group (1.50 ± 0.22 and 2.57 ± 0.20, $P = 0.011$; 1.57 ± 0.30 and 2.57 ± 0.20, $P = 0.025$, respectively). The PDGFR beta scores of the control and nilotinib groups were similar ($P > 0.05$) (Figure 3).

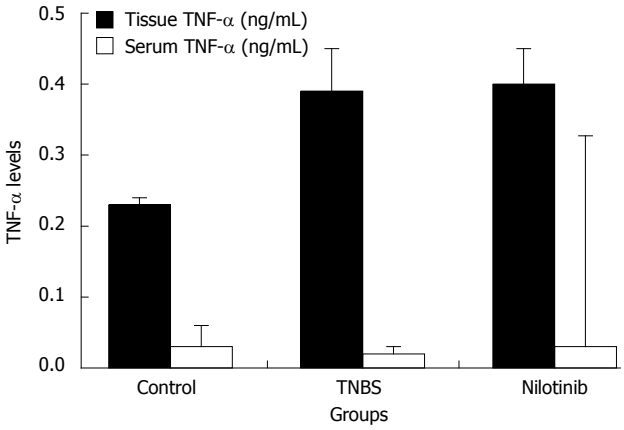

Figure 4 Tissue and serum tumor necrosis factor α levels among the experimental groups. The results are the mean ± SD. Serum tumor necrosis factor (TNF) and tissue TNF α levels were similar between the trinitrobenzene sulfonic acid (TNBS) and nilotinib groups.

The mean serum TNF alpha levels in the control, TNBS, and nilotinib groups were 0.03 ± 0.03, 0.02 ± 0.01, and 0.03 ± 0.01 pg/mL, respectively. There was no statistically significant difference observed among the groups in terms of the mean serum TNF alpha levels ($P > 0.05$) (Figure 4). The average tissue TNF alpha levels in the control, TNBS, and nilotinib groups were 0.23 ± 0.01, 0.39 ± 0.06, and 0.40 ± 0.05 ng/mL, respectively. There was a significant difference observed among the groups ($P = 0.002$). TNF alpha levels were significantly lower in the control group than in the TNBS or nilotinib groups (0.23 ± 0.01 and 0.39 ± 0.06 ng/mL, $P = 0.002$; 0.23 ± 0.01 and 0.40 ± 0.05 ng/mL, $P = 0.003$, respectively). However, there was no statistically significant difference between the TNBS and nilotinib groups in terms of the mean tissue TNF alpha levels ($P > 0.05$) (Figure 4).

The mean number of apoptotic cells detected by the TUNEL method in the control, TNBS, and nilotinib groups was 5.50 ± 0.67, 4.14 ± 0.88, and 4.14 ± 1.06, respectively. The difference among the groups was not statistically significant ($P > 0.05$) (Figure 5).

DISCUSSION

IBDs, such as CD and UC, are chronic recurrent intestinal inflammatory conditions. Genetic, environmental, microbial, and immune factors play a role in the etiopathogenesis of IBDs. Despite the development of biological therapies and advancements in genetic technology, treatment options remain limited for refractory cases.

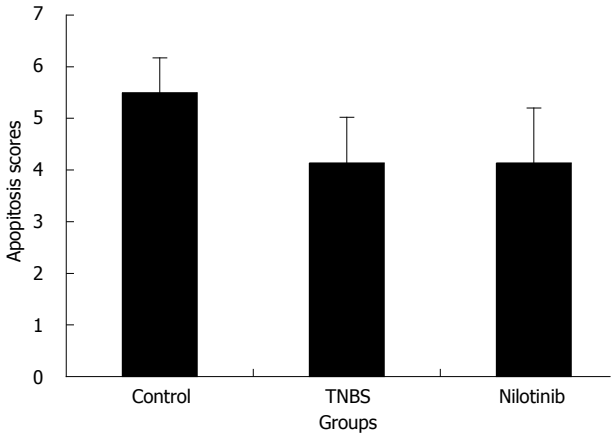

Figure 5 Apoptosis scores among the experimental groups. The results are the mean ± SD. Apoptosis scores were similar among the groups. TNBS: Trinitrobenzene sulfonic acid.

Mucosal healing has emerged as a key treatment goal for IBD and allows the prediction of sustained clinical remission and resection-free survival in affected patients. Mucosal healing can be achieved in approximately 30% of patients receiving corticosteroid therapy and in 60% of patients receiving anti-TNF agents[9-12]. Approximately 20% of IBD patients do not respond to anti-TNF therapy and require surgical intervention[12]. Thus, the currently available medical treatment options are ineffective in a substantial group of patients with IBD.

Nilotinib, which was used in this study, is a strong TK inhibitor that was initially approved for use in patients with imatinib-intolerant and imatinib-resistant Philadelphia chromosome-positive chronic or accelerated phase-CML and has since been approved as a frontline therapy in chronic phase CML[19]. Nilotinib is more potent than imatinib, which inhibits the autophosphorylation of various kinases, such as BCR-ABL, PDGFR, and c-KIT[19]. Nilotinib is generally well tolerated. Due to the lack of Src family kinase inhibition, myelosuppression is an infrequent adverse event that occurs less frequently with nilotinib than with other TK inhibitors[19]. The most common manageable adverse events are rash, pruritus, fatigue and headache. Neutropenia, anemia, thrombocytopenia, elevations of liver enzymes, cardiac toxicity, namely QT prolongation, fluid retention, edema, and weight gain are among the less common side effects[19]. TK inhibitors affect several key components in the pathogenesis of IBD, including TNF alpha, PDGFR, and NO synthesis. In this study, we evaluated the efficacy of nilotinib on weight, macroscopic and microscopic pathological scores, TNF alpha and PDGFR levels, and the apoptotic index in rat models with TNBS-induced colitis. There are no previous reports in the literature evaluating the efficacy of nilotinib in either a rat model of colitis or in human colitis.

In the present study, the weights of the control and experimental rats were monitored daily. At the end of 14 d, rats in the nilotinib group had lost significantly less weight than rats in the TNBS group ($P = 0.047$). These results are similar to those obtained in the study by Cuzzocrea *et al*[16], in which weight loss was significantly reduced by 7 d of treatment with the TK inhibitor tyrphostin AG126 in a DNBS-induced colitis animal model. The TK inhibitor used in the study by Cuzzocrea *et al*[16] is different from that used in our study. However, our study indicates that nilotinib does have a positive effect on weight in animal models with colitis.

The first therapeutic target in drug studies for the treatment of IBD was the regression of disease-related symptoms. The most important reason for this was that the agents used in the treatment of IBD were not disease-modifying drugs. In more recent studies, however, the primary endpoint in evaluating the therapeutic efficacy of drugs used to treat IBD has been "mucosal healing"[20]. With mucosal healing as the therapeutic target, continuous clinical remission and survival without surgery can be achieved[21]. The mucosal healing rates of anti-TNF agents have been reported at approximately 60% in the active ulcerative colitis trials (ACT)-1 and ACT-2 studies[7,10]. In the present study, the effects of nilotinib on mucosal healing and pathological macroscopic and microscopic scores yielded quite remarkable results. The macroscopic and microscopic pathological scores of intestinal tissue from the nilotinib group were similar to those of the control group ($P > 0.05$) but significantly lower than those of the TNBS group ($P = 0.009$; $P = 0.030$, respectively). In our study, the similar microscopic and macroscopic scores of the nilotinib and control groups constituted the most important evidence of the mucosal healing effect of nilotinib. Indeed, in the study conducted by Cuzzocrea *et al*[16], the rats treated with the TK inhibitor showed significant histological improvements after treatment compared with the control rats. This result parallels the results of our study. Although there are no human studies investigating the use of TK inhibitors in patients with IBD, the case report by Magro *et al*[15] representing a case of long-standing remission from Crohn's disease under imatinib therapy supports these results. However, a number of caveats should be noted regarding the present study. This is the first study of nilotinib in a rat colitis model. The current study was unable to compare the efficacy of nilotinib with that of other IBD agents or to assess the adverse events of nilotinib. Generally, as found in previous studies on CML, nilotinib has been a well-tolerated agent with manageable adverse effects. The findings of this study have a number of important implications for future practice. Further experimental investigations could provide more definitive evidence for human studies.

In our study, to determine the effectiveness of nilotinib on colitis, the PDGFR alpha, PDGFR beta, TNF alpha, and apoptosis levels were compared among the groups. Similar to the results observed in the macroscopic and microscopic pathological scores, the PDGFR alpha and beta scores were significantly lower in the nilotinib group than in the TNBS group ($P = 0.014$, $P = 0.025$) but were similar to the control group. There are no other studies investigating the effects of TK inhibitors on the levels of PDGFR alpha and beta in a colitis animal model. Histologically, in IBD, the intestinal microvascula-

ture shifts into a tight angiogenic structure characterized by the increased secretion of angiogenic integrins and mediators into the inflamed mucosa[22]. PDGF alpha and beta are 2 of the angiogenic mediators whose levels increase in IBD[23]. Increased PDGF alpha and beta activity can be found in the fibrotic areas adjacent to the active ulcer areas in IBD. Kumagai *et al*[24] detected the increased expression of PDGF and PDGFR in the areas with active fibrosis in IBD, and they considered that this contributes to the development of IBD. The macrovascular results of this process were demonstrated as endothelial dysfunction in study of Principi *et al*[25] due to decreased brachial artery flow-mediated vasodilatation in patients with IBD. The results of our study suggest that nilotinib enacts its effect on mucosal healing in colitis by blocking PDGFR alpha and beta.

TNF alpha is a protein that plays a role in cell proliferation, differentiation, and cell survival. It is responsible for the expression of adhesion molecules, fibroblast proliferation, the release of procoagulant substances, the initiation of cytotoxic apoptosis, and the acute phase response[26]. It has a clearly defined role in the pathogenesis of IBD, and anti-TNF agents are currently being used in the successful treatment of IBD[10]. In IBD, induced apoptosis can be triggered by TNF alpha, which causes much larger leaks in the intestinal barrier[27]. Previous studies have demonstrated that TNF alpha and IL-1 beta, both proinflammatory cytokines synthesized in the colon, are reduced with TK inhibition[16,28]. In our study, the apoptotic indices and serum and tissue levels of TNF alpha were evaluated. The serum and tissue levels of TNF alpha and the apoptotic index in the nilotinib group were found to be similar to those in the TNBS group. Previously, it has been shown that TNF alpha levels on day 7 are significantly higher in acute models of colitis established through a single dose application of TNBS, compared to the model of chronic colitis using weekly TNBS administrations[29,30]. That the serum and tissue TNF alpha levels were similar in the nilotinib and TNBS groups in our study might be explained by the length of the experiment (14 d), during which a TNF alpha peak could not be obtained. Additionally, the apoptosis indices were similar between both groups in our study. D'Argenio *et al*[31] demonstrated the apoptotic cells and expressions of apoptotic proteins in TNBS-induced colitis over 4 wk. According to the results of this study, the apoptotic cell count was detected to be significantly decreased after first week by the TUNEL method[31]. The similar apoptotic scores detected in our study might be because the apoptotic cell peak could not be obtained after 14 d. Furthermore, the similar results of the TNF alpha levels and apoptosis scores in our study might also suggest that nilotinib has no significant effect on TNF alpha levels and apoptosis.

In conclusion, nilotinib has a significant effect on weight loss, as well as on the macroscopic and microscopic pathological scores in rats with TNBS-induced colitis, leading to significant mucosal healing. Although nilotinib caused a decrease in PDGFR alpha and PDGFR beta levels, it did not have a significant effect on apoptotic scores or TNF alpha levels.

COMMENTS

Background

Genetic and environmental factors, infectious agents, the structure of enteric flora, and immune system dysfunction are key elements in the pathogenesis of inflammatory bowel disease (IBD); thus, these are the targets for many drugs developed to treat IBD. Unresponsiveness to medical treatments in refractory IBD still poses a therapeutic challenge. Tyrosine kinases (TKs) are enzymes that play a role in normal cell function, metabolism, growth, differentiation, and apoptosis. To establish an alternative treatment option, they selected nilotinib based on the fact that TK inhibitors affect several key components in the pathogenesis of IBD, including tumor necrosis factor (TNF) alpha, platelet-derived growth factor receptor (PDGFR), and apoptosis.

Research frontiers

Nilotinib is a TK inhibitor that is typically used as an anticancer drug. Recently, it has been considered for use in noncancerous proliferative diseases and for inflammatory conditions. Authors concluded that nilotinib has a significant effect on weight loss and macroscopic and microscopic pathological scores while leading to significant mucosal healing. Although nilotinib caused a decrease in the PDGFR alpha and PDGFR beta levels, it did not have a significant effect on apoptotic scores or TNF alpha levels.

Innovations and breakthroughs

Genetic, environmental, microbial, and immune factors play a role in the etiopathogenesis of IBDs. The currently available medical treatment options are ineffective in a substantial group of patients with IBD. Nilotinib, as used in this study, is a strong TK inhibitor. TK inhibitors affect several key components in the pathogenesis of IBD, including TNF alpha, PDGFR, and nitric oxide synthesis. Before this study, there were no reports in the literature evaluating the efficacy of nilotinib in a rat colitis model. One previous study showed that the use of a different TK inhibitor could successfully treat rat colitis. There are no human studies investigating the use of TK inhibitors in patients with IBD.

Applications

The results of this study suggest that nilotinib has a significant effect on weight loss, as well as on the macroscopic and microscopic pathological scores of rats with Trinitrobenzene sulfonic acid (TNBS)-induced colitis. Additionally, this treatment leads to significant mucosal healing and caused a decrease in PDGFR alpha and PDGFR beta levels, although it did not have a significant effect on apoptotic scores or TNF alpha levels. These results suggest that *nilotinib* may be effective in patients with IBD. The findings of this study have a number of important implications for the future practice. Therefore, further studies are needed to draw firm conclusions.

Terminology

IBD are chronic inflammatory disorders of the gastrointestinal tract that have characteristic clinical, pathological, endoscopic and radiological features. TKs are enzymes that play a role in normal cell function, metabolism, growth, differentiation, and apoptosis. TK inhibitors are drugs that block the action of these enzymes. TNBS-induced colitis is a well-established animal model of mucosal inflammation that has been used for over 2 decades in the study of IBD pathogenesis, as well as in preclinical studies.

Peer review

This is an excellent basic research animal study using a well-known model of TNBS-induced colitis in rats. The authors explored the ability of a tyrosine kinase inhibitor, nilotinib, to treat various clinical (weight determination), laboratory (TNF levels, apoptotic index) and pathological parameters (macroscopic and microscopic pathologic scores, PDGFR levels) and to quantify results. They were basically able to demonstrate mucosal healing effects, clinical improvements and decreased PDGFR alpha and beta levels; however, significant drops in the TNF peaks and apoptotic indices were not clearly shown.

REFERENCES

1 **Bosani M**, Ardizzone S, Porro GB. Biologic targeting in the treatment of inflammatory bowel diseases. *Biologics* 2009; **3**: 77-97 [PMID: 19707398]

2 **Baumgart DC**, Carding SR. Inflammatory bowel disease:

cause and immunobiology. *Lancet* 2007; **369**: 1627-1640 [PMID: 17499605 DOI: 10.1016/S0140-6736(07)60750-8]
3 **Engel MA**, Neurath MF. New pathophysiological insights and modern treatment of IBD. *J Gastroenterol* 2010; **45**: 571-583 [PMID: 20213337 DOI: 10.1007/s00535-010-0219-3]
4 **Marshall JK**, Thabane M, Steinhart AH, Newman JR, Anand A, Irvine EJ. Rectal 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2012; **11**: CD004118 [PMID: 23152224 DOI: 10.1002/14641858]
5 **Sutherland L**, MacDonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2003; (3): CD000543 [PMID: 12917894 DOI: 10.1002/14651858.CD000543]
6 **Gisbert JP**, Linares PM, McNicholl AG, Maté J, Gomollón F. Meta-analysis: the efficacy of azathioprine and mercaptopurine in ulcerative colitis. *Aliment Pharmacol Ther* 2009; **30**: 126-137 [PMID: 19392869 DOI: 10.1111/j.1365-2036.2009.04023.x]
7 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]
8 **Reinisch W**, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]
9 **TRUELOVE SC**, WITTS LJ. Cortisone in ulcerative colitis; preliminary report on a therapeutic trial. *Br Med J* 1954; **2**: 375-378 [PMID: 13182220]
10 **Rutgeerts P**, Vermeire S, Van Assche G. Mucosal healing in inflammatory bowel disease: impossible ideal or therapeutic target? *Gut* 2007; **56**: 453-455 [PMID: 17369375 DOI: 10.1136/gut.2005.088732]
11 **Feagan BG**, Reinisch W, Rutgeerts P, Sandborn WJ, Yan S, Eisenberg D, Bala M, Johanns J, Olson A, Hanauer SB. The effects of infliximab therapy on health-related quality of life in ulcerative colitis patients. *Am J Gastroenterol* 2007; **102**: 794-802 [PMID: 17324131 DOI: 10.1111/j.1572-0241.2007.01094.x]
12 **Peyrin-Biroulet L**, Lémann M. Review article: remission rates achievable by current therapies for inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **33**: 870-879 [PMID: 21323689 DOI: 10.1111/j.1365-2036.2011.04599.x]
13 **Manley PW**, Stiefl N, Cowan-Jacob SW, Kaufman S, Mestan J, Wartmann M, Wiesmann M, Woodman R, Gallagher N. Structural resemblances and comparisons of the relative pharmacological properties of imatinib and nilotinib. *Bioorg Med Chem* 2010; **18**: 6977-6986 [PMID: 20817538 DOI: 10.1016/j.bmc.2010.08.026]
14 **Rhee CK**, Lee SH, Yoon HK, Kim SC, Lee SY, Kwon SS, Kim YK, Kim KH, Kim TJ, Kim JW. Effect of nilotinib on bleomycin-induced acute lung injury and pulmonary fibrosis in mice. *Respiration* 2011; **82**: 273-287 [PMID: 21659722 DOI: 10.1159/000327719]
15 **Magro F**, Costa C. Long-standing remission of Crohn's disease under imatinib therapy in a patient with Crohn's disease. *Inflamm Bowel Dis* 2006; **12**: 1087-1089 [PMID: 17075351]
16 **Cuzzocrea S**, McDonald MC, Mazzon E, Mota-Filipe H, Lepore V, Ciccolo A, Terranova ML, Britti D, Caputi AP, Thiemermann C. The tyrosine kinase inhibitor tyrphostin AG 126 reduced the development of colitis in the rat. *Lab Invest* 2000; **80**: 1439-1453 [PMID: 11005212 DOI: 10.1038/labinvest.3780151]
17 **Vilaseca J**, Salas A, Guarner F, Rodríguez R, Martínez M, Malagelada JR. Dietary fish oil reduces progression of chronic inflammatory lesions in a rat model of granulomatous colitis. *Gut* 1990; **31**: 539-544 [PMID: 2161781 DOI: 10.1136/gut.31.5.539]
18 **Dieleman LA**, Palmen MJ, Akol H, Bloemena E, Peña AS, Meuwissen SG, Van Rees EP. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin Exp Immunol* 1998; **114**: 385-391 [PMID: 9844047 DOI: 10.1046/j.1365-2249.1998.00728.x]
19 **Deremer DL**, Katsanevas K, Ustun C. Critical appraisal of nilotinib in frontline treatment of chronic myeloid leukemia. *Cancer Manag Res* 2011; **3**: 65-78 [PMID: 21556318 DOI: 10.2147/CMR.S11948]
20 **Chevaux JB**, Vavricka SR, Rogler G, Lakatos PL, Schoepfer A, Peyrin-Biroulet L. Mucosal healing with anti-TNF antibodies. *Digestion* 2012; **86** Suppl 1: 16-22 [PMID: 23051722 DOI: 10.1159/000341957]
21 **Neurath MF**, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. *Gut* 2012; **61**: 1619-1635 [PMID: 22842618 DOI: 10.1136/gutjnl-2012-302830]
22 **Danese S**, Sans M, Spencer DM, Beck I, Doñate F, Plunkett ML, de la Motte C, Redline R, Shaw DE, Levine AD, Mazar AP, Fiocchi C. Angiogenesis blockade as a new therapeutic approach to experimental colitis. *Gut* 2007; **56**: 855-862 [PMID: 17170016 DOI: 10.1136/gut.2006.114314]
23 **Cromer WE**, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. *World J Gastroenterol* 2011; **17**: 578-593 [PMID: 21350707 DOI: 10.3748/wjg.v17.i5.578]
24 **Kumagai S**, Ohtani H, Nagai T, Funa K, Hiwatashi NO, Shimosegawa H. Platelet-derived growth factor and its receptors are expressed in areas of both active inflammation and active fibrosis in inflammatory bowel disease. *Tohoku J Exp Med* 2001; **195**: 21-33 [PMID: 11780721 DOI: 10.1620/tjem.195.21]
25 **Principi M**, Mastrolonardo M, Scicchitano P, Gesualdo M, Sassara M, Guida P, Bucci A, Zito A, Caputo P, Albano F, Ierardi E, Di Leo A, Ciccone MM. Endothelial function and cardiovascular risk in active inflammatory bowel diseases. *J Crohns Colitis* 2013; **7**: e427-e433 [PMID: 23473915 DOI: 10.1016/j.crohns.2013.02.001]
26 **Műzes G**, Molnár B, Tulassay Z, Sipos F. Changes of the cytokine profile in inflammatory bowel diseases. *World J Gastroenterol* 2012; **18**: 5848-5861 [PMID: 23139600 DOI: 10.3748/wjg.v18.i41.5848]
27 **Gitter AH**, Bendfeldt K, Schulzke JD, Fromm M. Leaks in the epithelial barrier caused by spontaneous and TNF-alpha-induced single-cell apoptosis. *FASEB J* 2000; **14**: 1749-1753 [PMID: 10973924 DOI: 10.1096/fj.99-0898com]
28 **De Backer O**, Lefebvre RA. Is there a role for imatinib in inflammatory bowel disease? *Inflamm Bowel Dis* 2008; **14**: 579-581 [PMID: 18050299 DOI: 10.1002/ibd.20320]
29 **Fitzpatrick LR**, Meirelles K, Small JS, Puleo FJ, Koltun WA, Cooney RN. A new model of chronic hapten-induced colitis in young rats. *J Pediatr Gastroenterol Nutr* 2010; **50**: 240-250 [PMID: 20118800 DOI: 10.1097/MPG.0b013e3181cb8f4a]
30 **Zhu MY**, Lu YM, Ou YX, Zhang HZ, Chen WX. Dynamic progress of 2,4,6-trinitrobenzene sulfonic acid induced chronic colitis and fibrosis in rat model. *J Dig Dis* 2012; **13**: 421-429 [PMID: 22788928 DOI: 10.1111/j.1751-2980.2012.00607.x]
31 **D'Argenio G**, Farrace MG, Cosenza V, De Ritis F, Della Valle N, Manguso F, Piacentini M. Expression of apoptosis-related proteins in rat with induced colitis. *Int J Colorectal Dis* 2004; **19**: 451-460 [PMID: 15083321 DOI: 10.1007/s00384-004-0585-5]

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BRIEF ARTICLE

Lymph node metastasis in gastric cardiac adenocarcinoma in male patients

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Abstract

AIM: To reveal the clinicopathological features and risk factors for lymph node metastases in gastric cardiac adenocarcinoma of male patients.

METHODS: We retrospective reviewed a total of 146 male and female patients with gastric cardiac adenocarcinoma who had undergone curative gastrectomy with lymphadenectomy in the Department of Surgery, Xin Hua Hospital and Rui Jin Hospital of Shanghai Jiaotong University Medical School between November 2001 and May 2012. Both the surgical procedure and extent of lymph node dissection were based on the recommendations of Japanese gastric cancer treatment guidelines. Univariate and multivariate analyses of lymph node metastases and the clinicopathological features were undertaken.

RESULTS: The rate of lymph node metastases in male patients with gastric cardiac adenocarcinoma was 72.1%. Univariate analysis showed an obvious correlation between lymph node metastases and tumor size, gross appearance, differentiation, pathological tumor depth, and lymphatic invasion in male patients. Multivariate logistic regression analysis revealed that tumor differentiation and pathological tumor depth were the independent risk factors for lymph node metastases in male patients. There was an obvious relationship between lymph node metastases and tumor size, gross appearance, differentiation, pathological tumor depth, lymphatic invasion at pN_1 and pN_2, and nerve invasion at pN_3 in male patients. There were no significant differences in clinicopathological features or lymph node metastases between female and male patients.

CONCLUSION: Tumor differentiation and tumor depth were risk factors for lymph node metastases in male patients with gastric cardiac adenocarcinoma and should be considered when choosing surgery.

Key words: Gastric neoplasm; Lymph node metastasis; Risk factors; Gastrectomy; Lymphadenectomy

Core tip: There is an obvious correlation between lymph node metastases and tumor size, gross appearance, differentiation, pathological tumor depth and lymphatic invasion in male patients. Tumor differentiation and

pathological tumor depth were independent risk factors for lymph node metastases in male patients. There was an obvious relationship between lymph node metastases and tumor size, gross appearance, differentiation, pathological tumor depth, lymphatic invasion at pN_1 and pN_2, and nerve invasion at pN_3 in male patients. There were no significant differences in clinicopathological features or lymph node metastases between female and male patients.

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INTRODUCTION

Although its incidence and mortality have declined over the past 50 years, gastric cancer (GC) remains the fourth most common cancer and the second most frequent cause of cancer death worldwide[1-3]. In China, GC is the second most common malignancy and the third most frequent cause of cancer-related death, with an annual age-adjusted mortality rate of 24.34 deaths per 100000 people[4]. As GC incidence declines, the frequency of proximal gastric and gastroesophageal junctional adenocarcinomas continues to rise, and has become a significant clinical challenge[5-7]. The reasons for this rapid increase in aggressive proximal malignancies remain unclear. Tumors in the upper third of the stomach might spread *via* the lymphatic system through the lower esophageal channel to the mediastinum, through the suprapancreatic channel to the abdomen, or through the abdominal para-aortic channel to the retroperitoneum. Surgery is currently the only treatment that can lead to a cure. However, the optimal surgical strategy for tumors in the cardiac area of the stomach, especially tumors invading the lower esophagus, remains controversial[6]. The development of effective therapeutic strategies for these tumors requires information on patient characteristics, patterns of lymph node metastasis, and the efficacy of lymph node dissection. Adenocarcinoma of the cardia generally has a low curative resection rate and a poor prognosis; worse than carcinoma of the other regions of the stomach, mainly because the disease is at a more advanced stage at diagnosis[6-8]. The 5-year survival rate in resected cases is $\leqslant$ 20%[9].

The role of lymphadenectomy in GC surgery has been hotly debated during the past three decades. Although there is still no standard approach, it is obvious that an adequate lymphadenectomy, removing all the possible metastatic nodes, remains a milestone in GC surgery[10]. The most recent edition of the tumor, node, metastasis (TNM) classification states that at least 15 lymph nodes must be examined to form an accurate evaluation of the node status. The optimal extent of lymphadenectomy (D2) for this cancer has been defined in the Japanese Classification of Gastric Carcinoma[11], based on the retrospective historical data of the involved nodes in patients with gastric carcinoma. The optimal extent of lymph node dissection for Siewert type Ⅱ esophagogastric junction (EGJ) carcinoma is poorly defined in this classification. Rüdiger Siewert *et al*[12] uncovered the distribution of metastatic nodes in patients with type Ⅱ adenocarcinoma. In their cohort of 186 patients, they found that the disease mainly involved the paracardial and lesser curve nodes, followed in frequency by the nodes in the lower mediastinum, and suprapancreatic nodes and nodes along the greater curve were involved in patients with Siewert type Ⅱ EGJ cancers. Furthermore, they found positive parapyloric nodes in three of their patients, which lends support to their recommended strategy of extended total gastrectomy for type Ⅱ EGJ carcinoma.

Therefore, in the present study, we revaluated retrospectively the clinicopathological features and distribution of metastatic nodes in a two-center cohort of 146 patients with gastric cardiac adenocarcinoma. Univariate and multivariate analyses were applied to confirm the clinicopathological factors associated with lymph node metastases, and to provide a basis for choosing the optimal surgical treatment and for determining the appropriate range of lymph node dissection.

MATERIALS AND METHODS

Patients

Data were collected from a prospectively maintained database of patients with histologically confirmed gastric cardiac carcinoma who had curative gastrectomy (R0) with lymphadenectomy in the Department of Surgery, Xin Hua Hospital and Rui Jin Hospital of Shanghai Jiaotong University Medical School between November 2001 and May 2012. The clinicopathological characteristics and lymph node metastasis of gastric cardiac adenocarcinoma were compared in male and female patients (Table 1).

Surgery

All operations were performed with curative intent. Curative surgery was defined as the removal of all gross tumor and the demonstration of tumor-negative surgical margins by microscopic examination of the entire circumference. Subtotal or total gastrectomy was performed according to the tumor size, tumor location, and the status of the resection margins. Proximal gastrectomy involved resection of the proximal half of the stomach *via* an abdominal or thoracic approach, with an esophagogastric anastomosis. Following total gastrectomy with D2 lymph node dissection, an esophagojejunostomy was used routinely for Roux-en-Y reconstruction. Proximal resection margins were evaluated intraoperatively to confirm freedom from disease. Resection of adjacent organs was undertaken to achieve clear margins when deemed necessary. Both the surgical procedure and the extent of

Table 1 Demographics and clinicopathological features of gastric cardiac adenocarcinoma

Factors	Total (n = 146)	Sex		P value
		Female (n = 35)	Male (n = 111)	
Age (yr)				0.668
< 60	46	10	36	
⩾ 60	100	25	75	
Type of gastrectomy				0.776
Total	53	12	41	
Proximal gastrectomy	93	23	70	
Splenectomy				0.102
Presence	8	0	8	
Absence	138	35	103	
Tumor size (cm)				0.717
< 2	2	0	2	
2-5	93	23	70	
> 5	51	12	39	
Gross appearance				0.931
Type 0	6	2	4	
Type 1	13	3	10	
Type 2	105	26	79	
Type 3	13	2	11	
Type 4	9	2	7	
Tumor differentiation				0.389
Differentiated	76	16	60	
Undifferentiated	70	19	51	
Lymph nodes retrieved	22.88 ± 9.162	23.06 ± 9.449	22.78 ± 9.089	0.602
Pathological tumor depth				0.729
T1	8	2	6	
T2	16	2	14	
T3	4	1	3	
T4	118	30	88	
Node status (TNM)				0.665
pN_0	43	11	32	
pN_1	27	4	23	
pN_2	29	8	21	
pN_3	47	12	35	
pTNM staging				0.445
Ⅰ	15	2	13	
Ⅱ	37	11	26	
Ⅲ	94	22	72	
Ⅳ				
Lymphatic invasion				0.694
Positive	24	5	19	
Negative	122	30	92	
Venous invasion				0.393
Positive	5	2	3	
Negative	141	33	108	
Nerve invasion				0.350
Positive	22	7	15	
Negative	124	28	96	
Esophageal involvement				0.497
Presence	31	6	25	
Absence	115	29	86	

TNM: Tumour, node, metastasis.

lymph node dissection were based on the recommendations of the Japanese GC treatment guidelines[13]. No patient received neoadjuvant chemotherapy or postoperative radiotherapy.

Pathological examination

In both hospitals, the surgical team immediately examined the lymph nodes macroscopically, which were then divided and classified into lymph node stations, as defined by the Japanese Classification of Gastric Carcinoma[14]. No size limitation was imposed for lymph node harvesting. Specimens were fixed in formalin, stained with hematoxylin and eosin, and sent for histopathological evaluation, following which the number of histologically confirmed lymph nodes was recorded for each lymph node station. Each lymph node was embedded in paraffin and at least two sections were taken. Immunhistochemistry for micrometastasis was not performed.

Tumor size was recorded as the maximum diameter. The depth of infiltration was measured at the deepest point of penetration of the cancer cells. In this study, we referred to the classifications established by the Japanese Classification of Gastric Carcinoma: 3rd English edition[14], which define T1 as a tumor confined to the mucosa (M) or submucosa (SM); T2 as a tumor that invades the muscularis propria (MP); T3 as a tumor that invades the subserosa (SS); and T4 as tumor invasion that is contiguous to or exposed beyond the serosa (SE) or tumor invades adjacent structures (SI). The macroscopic type was classified as type 0 (superficial), type 1 (mass), type 2 (ulcerative), type 3 (infiltrative ulcerative), type 4 (diffuse infiltrative) or type 5 (unclassifiable). We evaluated the tumor histology according to the classification established by the Japanese Research Society for GC[11]. Well-and moderately differentiated tubular adenocarcinoma, and papillary adenocarcinoma were classified as differentiated-type carcinomas; and poorly differentiated adenocarcinoma, signet ring cell carcinoma and mucinous carcinoma were classified as undifferentiated-type carcinomas.

The nodal classification was classified into four groups: pN_0, no metastasis; pN_1, one or two positive regional lymph nodes; pN_2, 3-6 positive regional lymph nodes; and pN_3, ⩾ 7 positive regional lymph nodes. We conducted the tumor staging according to the Japanese Classification of Gastric Carcinoma: 3rd English edition[14]. The ratio of lymph node metastasis was calculated by determining the number of patients with a metastasized lymph node in a particular station divided by the number of patients who underwent dissection of that lymph node. The metastatic incidence is the ratio of metastatic nodes to the total number of dissected nodes and was recorded for each nodal station for all regional lymph nodes.

Ethics

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The Institutional Review Board of Shanghai Jiaotong University gave ethical approval for this study. All patients provided written informed consent.

Statistical analysis

Descriptive data are presented as the mean ± SD. For between group comparisons, continuous variables were analyzed using Student's t test, and categorical variables with the χ^2 test. Factors found to be significant (P <

Table 2 Univariable analysis of lymph nodes metastasis in gastric cardiac cancer and clinicopathological factors

Factors	Total (n = 146)			Female (n = 35)			Male (n = 111)		
	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value
Age (yr)			0.927			0.134			0.353
< 60	33	13		5	5		28	8	
⩾ 60	71	29		19	6		52	23	
Tumor size (cm)			0.011			0.554			0.011
< 2	0	2		0	0		0	2	
2-5	62	31		15	8		47	23	
> 5	42	9		9	3		33	6	
Gross appearance			0.000			0.211			0.000
Type 0	0	6		0	2		0	4	
Type 1	5	8		2	1		3	7	
Type 2	82	23		19	7		63	16	
Type 3	10	3		2	0		8	3	
Type 4	7	2		1	1		6	1	
Tumor differentiation			0.000			0.150			0.000
Differentiated	44	32		9	7		35	25	
Undifferentiated	60	10		15	4		45	6	
Pathological tumor depth			0.000			0.051			0.000
T1	1	7		0	2		1	5	
T2	8	8		2	0		6	8	
T3	3	1		0	1		3	0	
T4	92	26		22	8		70	18	
Lymphatic invasion			0.001			0.102			0.003
Positive	24	0		5	0		19	0	
Negative	80	42		19	11		61	31	
Venous invasion			0.659			0.324			0.832
Positive	4	1		2	0		2	1	
Negative	100	41		22	11		78	30	
Nerve invasion			0.966			0.856			0.972
Positive	15	6		5	2		11	4	
Negative	88	36		19	9		69	27	
Esophageal involvement			0.192			0.912			0.131
Presence	25	6		4	2		21	4	
Absence	79	36		20	9		59	27	

0.05) in univariate analysis were included in subsequent multivariate logistic regression analysis, to identify independent variables associated with lymph node metastases. All statistical analyses were undertaken using SPSS for Windows, version 18.0 (SPSS, Chicago, IL, United States). For all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Demographics and clinicopathological features of gastric cardiac adenocarcinoma

The clinicopathological characteristics of gastric cardiac cancer are illustrated in Table 1. Among the 146 patients, there were 111 men and 35 women, ranging in age from 16 to 84 years (mean 63.9 ± 11.6 years). Surgical procedures comprised 93 proximal gastrectomies and 53 total gastrectomies. Splenectomy was required in 8 (5.5%) of the 146 patients undergoing curative resections. The total splenectomy patients were all male. Mean tumor length was 5.54 cm. Of the 146 patients, 6 (4.1%), 13 (8.9%), 105 (71.9%), 13 (8.9%) and 8 (5.8%) were type 0, 1, 2, 3 and 4, respectively. Tumors were differentiated in 76 patients and undifferentiated in 70. The number of lymph nodes retrieved was 22.88 ± 9.16, and 104 patients had positive lymph node metastases (71.2%). There were eight cases with a T1 tumor, 16 with a T2 tumor, 4 with a T3 tumor tumor, and 118 with a T4 tumor. Lymph node involvement according to the Japanese Classification of Gastric Carcinoma: 3rd English edition[14] included 43 patients with N0 disease, 27 with N1 disease, and 76 with N2-3 disease (Table 2). Evidence of lymphatic invasion, venous invasion and neural invasion was seen in 24 (16.4%), 5 (3.4%) and 22 patients (15.1%), respectively, On pathological examination, the tumors of 31 patients (21.2%) were found to have invaded the lower esophagus. None of the clinicopathological factors, such as age, type of gastrectomy, tumor size, gross appearance, tumor differentiation, pathological tumor depth, node status, pTNM staging, lymphatic invasion, venous invasion, nerve invasion and esophagus involvement were different between male and female patients ($P > 0.05$).

Univariate analysis of lymph node metastasis in gastric cardiac cancer and clinicopathological factors

Univariate analysis was performed on the relationship between lymph node metastases and clinicopathological factors. The findings revealed a close relationship between

Table 3 Univariate analysis of lymph node metastases in gastric cardiac adenocarcinoma and clinicopathological factors for sex differance

Factors	Female LN+ (n = 24)	Male LN+ (n = 80)	P value
Age (yr)			0.191
< 60	15.20%	84.80%	
⩾ 60	26.80%	73.20%	
Tumor size (cm)			0.743
< 2	0.00%	0.00%	
2-5	24.20%	75.80%	
> 5	21.40%	78.60%	
Gross appearance			0.961
Type 0	0.00%	0.00%	
Type 1	40.00%	60.00%	
Type 2	23.20%	76.80%	
Type 3	20.00%	80.00%	
Type 4	14.30%	85.70%	
Tumor differentiation			0.587
Differentiated	20.50%	79.50%	
Undifferentiated	25.00%	75.00%	
Pathological tumor depth			0.627
T1	0.00%	100.00%	
T2	25.00%	75.00%	
T3	0.00%	100.00%	
T4	23.90%	76.10%	
Lymphatic invasion			0.766
Positive	20.80%	79.20%	
Negative	23.80%	76.20%	
Venous invasion			0.192
Positive	50.00%	50.00%	
Negative	22.00%	78.00%	
Nerve invasion			0.399
Positive	31.30%	68.70%	
Negative	21.60%	78.40%	
Esophageal involvement			0.335
Presence	16.00%	84.00%	
Absence	25.30%	74.70%	

tumor size, gross appearance, differentiation, pathological depth, lymphatic invasion and lymph node metastases in all patients (P = 0.011, P = 0.000, P = 0.000, P = 0.000 and P = 0.001, respectively) and in male patients (P = 0.011, P = 0.000, P = 0.000, P = 0.000 and P = 0.003, respectively). However, there was no obvious correlation between lymph node metastases and clinicopathological features in female patients, nor between male and female patients (Table 3).

Multivariate analysis of lymph node metastases in gastric cardiac cancer for the entire study population and male patients

Multivariate analysis revealed that only tumor differentiation was an independent risk factor for lymph node metastases in gastric cardiac cancer for the entire study population (P = 0.001). Tumor size, gross appearance, pathological depth and lymphatic invasion had no significant effect on nodal involvement rates (Table 4). Multivariate analysis revealed that tumor differentiation and pathological depth were independent risk factors for lymph node metastases in gastric cardiac cancer for male patients (P = 0.001, P = 0.020). Tumor size, gross appearance and lymphatic invasion had no significant effect on nodal involvement rates (Table 4).

Relationship between sex and number of metastatic lymph nodes

There was no significant difference between female and male patients in terms of the number of retrieved lymph nodes, using the independent sample t test (P = 0.878). The number of metastatic lymph nodes in female patients was higher than that in male patients (6.20 ± 7.49 *vs* 4.84 ± 5.44). However, the difference was not significant (P = 0.243).

Retrieved lymph nodes, lymph node metastases, lymph node metastasis ratios and incidence for involved lymph nodes at each station in gastric cardiac adenocarcinoma

Lymph nodes (n = 3340, median 22.88; range 15-62) were removed from the 146 patients and examined, and 754 (median 5.16; range 0-30) were metastatic. For female patients, 807 (median 23.06; range 15-61) lymph nodes were examined and 217 (median 6.20; range 0-30) contained metastases. For male patients, 2533 (median 22.82; range 15-62) lymph nodes were examined and 537 (median 4.84; range 0-26) contained metastases (Table 5).

According to the Japanese Classification of Gastric Carcinoma: 3rd English edition[14], 103 cases (70.5%) were at N1, 23 cases (15.8%) at N2, 15 cases (10.3%) at N3, and four cases (2.7%) at M. A direct skip to N3, without moving through N2, occurred in 10 cases (6.8%). There were no skips to N2 without going through N1. Nodal metastases were frequent in the abdominal nodes, followed in frequency by involvement of the No. 3 (59.6%), No. 1 (26.7%), No. 2 (18.5%), and No. 4 (16.4%) nodes, and thereafter by mediastinal lymph nodes, which were affected only in a small number in our series (No. 110, 0.7%). The frequency of the metastatic involvement of the supra- and infra-pyloric nodes was low (4.1% and 3.4%, respectively), and no cases with metastasis to Nos. 13-15 were found. Only four patients received station No. 16 lymph node dissection, and three of them had metastasis (Table 5).

The extent of metastases in female cases was as follows: 24 cases were at N1 (16.4%, 24/146), representing a metastatic rate of 68.6% (24/35); 5 cases were at N2 (3.4%, 5/146), with a metastastic rate of 14.3% (5/146); and 4 cases were found at N3 (2.7%, 4/146), with a metastatic rate of 11.4% (4/146). The extent of metastases in male patients was as follows: 79 cases (54.1%) occurred at N1, with a metastatic rate of 71.2%; 18 cases occurred at N2 (12.3%), with an incidence of 16.2%; and 11 cases occurred at N3 (7.5%), with an incidence of 9.9% (Table 5).

Correlation between lymph node metastases at pN_1, pN_2 and pN_3 and clinicopathological factors, using the Japanese GC association classification for the entire study population and between male and female patients

Univariate analysis of variance revealed a close relationship between tumor size, gross appearance, differentiation, pathological depth and lymphatic invasion and

Table 4 Multivariate analysis of lymph node metastases in gastric cardiac cancer for the entire study population

Multivariate analysis	B	SE	χ^2 value	*P* value	OR	95%CI	
						Lower	Upper
Entire study population							
Tumor size	0.010	0.528	0.000	0.985	0.010	0.359	2.843
Gross appearance	-0.169	1.166	0.021	0.885	0.845	0.086	8.302
Tumor differentiation	1.806	0.522	11.981	0.001	6.084	2.188	16.912
Tumor depth	0.464	0.299	2.400	0.121	1.590	0.884	2.858
Lymphatic invasion	20.207	7720.675	0.000	0.998	5.967E8	0.000	
Constant	-3.181	1.664	3.656	0.056	0.042		
Male patients							
Tumor size	0.594	0.707	0.705	0.401	1.810	0.453	7.233
Gross appearance	-1.420	1.442	0.969	0.325	0.242	0.014	4.085
Tumor differentiation	2.525	0.749	11.375	0.001	12.493	2.880	54.199
Tumor depth	0.838	0.359	5.448	0.020	2.313	1.144	4.676
Lymphatic invasion	20.295	8351.751	0.000	0.998	6.514E8	0.000	
Constant	-5.010	2.212	5.132	0.023	0.007		

Table 5 Number of retrieved lymph nodes, lymph node metastases, lymph node metastasis ratios, and incidence at each station

Node station	pN category	Number of dissected nodes			Number of metastasis nodes			Incidence of lymph node metastasis			Ratio of lymph node metastasis		
		T	F	M	T	F	M	T	F	M	T	F	M
No. 1	pN_1	448	111	337	100	31	69	22.30%	26.90%	20.50%	26.70%	31.40%	24.30%
No. 2	pN_1	249	67	182	54	12	42	21.70%	17.90%	23.10%	18.50%	20.00%	18.00%
No. 3	pN_1	1308	334	974	404	100	304	30.90%	29.90%	31.20%	59.60%	60.00%	59.50%
No. 4	pN_1	589	136	453	87	45	42	14.80%	33.10%	9.30%	16.40%	22.90%	14.40%
No. 5	pN_3	39	9	30	11	7	4	28.20%	77.80%	13.30%	4.10%	5.70%	3.60%
No. 6	pN_3	146	33	113	13	9	4	8.90%	27.30%	3.50%	3.40%	8.60%	1.80%
No. 7	pN_2	176	49	127	32	8	24	18.20%	16.30%	18.90%	11.60%	8.60%	12.60%
No. 8	pN_2	108	26	82	13	3	10	12.00%	11.50%	12.20%	4.10%	5.70%	3.60%
No. 9	pN_2	39	8	31	15	2	13	38.50%	25.00%	41.90%	4.10%	2.90%	4.50%
No. 10	pN_2	37	11	26	8	0	8	21.60%	0.00%	30.80%	1.40%	0.00%	1.80%
No. 11	pN_2	36	6	30	5	0	5	13.90%	0.00%	16.70%	2.70%	0.00%	3.60%
No. 12	pN_2	45	6	39	5	0	5	11.10%	0.00%	12.80%	1.40%	0.00%	1.80%
No. 13	M	8	0	8	0	0	0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
No. 14	M	30	2	28	0	0	0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
No. 15	M	13	5	8	0	0	0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
No. 16	M	32	0	32	6	0	6	18.80%	0.00%	18.80%	2.10%	0.00%	2.70%
No. 110	M	37	4	33	1	0	1	2.70%	0.00%	3.00%	0.70%	0.00%	0.90%
Total		3340	807	2533	754	217	537	22.70%	26.90%	21.30%			

lymph node metastases at pN_1 in all patients ($P = 0.020$, $P = 0.000$, $P = 0.000$, $P = 0.000$ and $P = 0.001$, respectively) and male patients ($P = 0.021$, $P = 0.000$, $P = 0.001$, $P = 0.001$ and $P = 0.002$, respectively) (Table 6). However, there was no obvious correlation between lymph node metastases and clinicopathological features in female patients (Table 6) and between male and female patients (Table 7).

There was obvious relationship between the lymphatic invasion and lymph node metastases at pN_2 in all patients ($P = 0.048$) and male patients ($P = 0.046$) (Table 6). There was no significant correlation between clinicopathological features and the presence of lymph node metastases at pN_2 in female patients (Table 6) nor between male and female patients (Table 7).

There was an obvious relationship between lymphatic invasion and nerve invasion and lymph node metastases at pN_3 in all patients ($P = 0.009$, $P = 0.001$) (Table 6). There was a significant correlation between lymphatic invasion in female patients (Table 6) and neural invasion in male patients (Table 6) and the presence of lymph node metastases at pN_3. There was no significant correlation between clinicopathological features and the presence of lymph node metastases at pN_3 between male and female patients (Table 7).

DISCUSSION

According to some clinicians, true carcinoma of the cardia may be considered a distinct clinical entity, with different biological behavior and a more aggressive natural history than subcardial gastric carcinoma[15-17]. Strangely enough, the location, extent and even the existence of the gastric cardia are controversial[18]. Anatomists have applied the term cardia to that part of the stomach that lies around the orifice of the tubular esophagus. The American Joint Committee on Cancer describes the EGJ as the first part of the stomach, which is located immediately below the diaphragm and is often called the cardia[19]. The definition of the cardia commonly employed in Japan is

Table 6 Correlation between lymph node metastases at pN_1, pN_2 and pN_3 and clinicopathological factors

Characterists	pN_1									pN_2									pN_3								
	Total			Female			Male			Total			Female			Male			Total			Female			Male		
	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value	LN+	LN-	*P* value
Age (yr)			0.830			0.134			0.287			0.066			0.541			0.082			0.670			0.179			0.769
< 60	33	13		5	5		28	8		11	35		2	8		9	27		4	42		0	10		4	32	
⩾ 60	70	30		19	6		51	24		12	88		3	22		9	66		11	89		4	21		7	68	
Tumor size (cm)			0.020			0.554			0.021			0.151			0.191			0.314			0.095			0.068			0.345
< 2.0	0	2		0	0		0	2		0	2		0	0		0	2		0	2		0	0		0	2	
2.0-5.0	62	31		15	8		47	23		11	82		2	21		9	61		6	87		1	22		5	65	
> 5.0	41	10		9	3		32	7		12	39		3	9		9	30		9	42		3	9		6	33	
Gross appearance			0.000			0.211			0.000			0.674			0.513			0.748			0.395			0.428			0.560
Type 0	0	6		0	2		0	4		0	6		0	2		0	4		0	6		0	2		0	4	
Type 1	5	8		2	1		3	7		1	12		0	3		1	9		0	13		0	3		0	10	
Type 2	81	24		19	7		62	17		19	86		4	22		15	64		13	92		3	23		10	69	
Type 3	10	3		2	0		8	3		2	11		1	1		1	10		2	11		1	1		1	10	
Type 4	7	2		1	1		6	1		1	8		0	2		1	6		0	9		0	2		0	7	
Tumor differentiation			0.000			0.150			0.001			0.071			0.782			0.054			0.324			0.855			0.215
Differentiated	44	32		9	7		35	25		8	68		2	14		6	54		6	70		2	14		4	56	
Undifferentiate	59	11		15	4		44	7		15	55		3	16		12	39		9	61		2	17		7	44	
Pathological tumor depth			0.000			0.051			0.001			0.360			0.808			0.406			0.265			0.861			0.363
T1	1	7		0	2		1	5		0	8		0	2		0	6		0	8		0	2		0	6	
T2	8	8		2	0		6	8		1	15		0	2		1	13		0	16		0	2		0	14	
T3	3	1		0	1		3	0		1	3		0	1		1	2		0	4		0	1		0	3	
T4	91	27		22	8		69	19		21	97		5	25		16	72		15	103		4	26		11	77	
Lymphatic invasion			0.001			0.102			0.002			0.048			0.693			0.046			0.009			0.030			0.074
Yes	24	0		5	0		19	0		7	17		1	4		6	13		6	18		2	3		4	15	
No	79	43		19	11		60	32		16	106		4	26		12	80		9	113		2	28		7	85	
Venous invasion			0.637			0.324			0.861			0.791			0.137			0.440			0.466			0.601			0.169
Yes	4	1		2	0		2	1		1	4		1	1		0	3		1	4		0	2		1	2	
No	99	42		22	11		77	31		22	119		4	29		18	90		14	127		4	29		10	98	
Nerve invasion			0.792			0.856			0.679			0.734			1.000			0.669			0.001			0.111			0.001
Yes	15	7		5	2		10	5		4	18		1	6		3	12		7	15		2	5		5	10	
No	88	36		19	9		69	27		19	105		4	24		15	81		8	116		2	26		6	90	
Esophageal involvement			0.165			0.912			0.108			0.109			0.272			0.205			0.587			0.658			0.691
Yes	25	6		4	2		21	4		2	29		0	6		2	23		4	27		1	5		3	22	
No	78	37		20	9		58	28		21	94		5	24		16	70		11	104		3	26		8	78	

the area within 2 cm above and below the EGJ, and tumors whose center is situated in this area are considered to be cancer of the cardia; such cancers are distinguished from upper GCs. Siewert *et al*[20] proposed a topographical classification for cardiac carcinomas. In contrast to the previously described classification system, Siewert and Stein[20] attempted to resolve the problem of splitting up EGJ tumors into esophageal and gastric tumors by creating a third entity. These tumours show a high rate of early lymphatic dissemination and lymph node metastases[12,15,21] and are usually related to poorer prognosis[12,22]. The reasons for this sudden increase of gastric cardia carcinomas are not clear, but changing risk factors such as smoking, alcohol use, presentation at a more advanced stage, salty foods, pollution and increases in gastroesophageal reflux diseases might explain it partially[5,7,23].

Table 7 Correlation between lymph node metastases at pN1, pN2 and pN3 and clinicopathological factors for sex difference

Clinicopathological factors	pN1			pN2			pN3		
	Female LN+	Male LN+	*P* value	Female LN+	Male LN+	*P* value	Female LN+	Male LN+	*P* value
Age (yr)			0.179			0.692			0.159
< 60	5 (15.2)	28 (84.8)		2 (18.2)	9 (81.8)		0 (0.0)	4 (100.0)	
⩾ 60	19 (27.1)	51 (72.9)		3 (25.0)	9 (75.0)		4 (36.4)	7 (63.6)	
Tumor size (cm)			0.792			0.692			0.475
< 2.0	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
2.0-5.0	15 (24.2)	47 (75.8)		2 (18.2)	9 (81.8)		1 (16.7)	5 (83.3)	
> 5.0	9 (22.0)	32 (78.0)		3 (25.0)	9 (75.0)		3 (33.3)	6 (66.7)	
Gross appearance			0.960			0.472			0.423
Type 0	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Type 1	2 (40.0)	3 (60.0)		0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)	
Type 2	19 (23.5)	62 (76.5)		4 (21.1)	15 (78.9)		3 (23.1)	10 (76.9)	
Type 3	2 (20.0)	8 (80.0)		1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)	
Type 4	1 (14.3)	6 (85.7)		0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)	
Tumor differentiate			0.555			0.782			0.634
Differentiated	9 (20.5)	35 (79.5)		2 (25.0)	6 (75.0)		2 (33.3)	4 (66.7)	
Undifferentiate	15 (25.4)	44 (74.6)		3 (20.0)	12 (80.0)		2 (22.2)	7 (77.8)	
Pathological tumor depth			0.624			0.738			
T1	0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	NS
T2	2 (25.0)	6 (75.0)		0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)	
T3	0 (0.0)	3 (100.0)		0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)	
T4	22 (24.2)	69 (75.8)		5 (23.8)	16 (76.2)		4 (26.7)	11 (73.3)	
Lymphatic invasion			0.744			0.567			0.634
Positive	5 (20.8)	19 (79.2)		1 (14.3)	6 (85.7)		2 (33.3)	4 (66.7)	
Negative	19 (24.1)	60 (75.9)		4 (25.0)	12 (75.0)		2 (22.2)	7 (77.8)	
Venous invasion			0.198			0.052			0.533
Positive	2 (50.0)	2 (50.0)		1 (100.0)	0 (0.0)		0 (0.0)	1 (100.0)	
Negative	22 (22.2)	77 (77.8)		4 (18.2)	18 (81.8)		4 (28.6)	10 (71.4)	
Nerve invasion			0.247			0.862			0.733
Positive	5 (35.7)	9 (64.3)		1 (25.0)	3 (75.0)		2 (33.3)	4 (66.7)	
Negative	19 (21.6)	69 (78.4)		4 (21.1)	15 (78.9)		2 (25.0)	6 (75.0)	
Esophageal involvement			0.321			0.435			0.930
Presence	4 (16.0)	21 (84.0)		0 (0.0)	2 (100.0)		1 (25.0)	3 (75.0)	
Absence	20 (25.6)	58 (74.4)		5 (23.8)	16 (76.2)		3 (27.3)	8 (72.7)	

It is crucial that the therapeutic strategy for gastric cardiac adenocarcinoma be clarified through evaluation of both the pattern of lymph node metastasis and the efficacy of lymph node dissection in this region. According to Siewert *et al*[20], metastases already exist in 72.0% of the cases at the time of surgery on tumors of the distal esophagus and the cardia. Lymphogenous metastases were present in 73.5% of the cases in our study. The cause of the frequent invasion of lymph nodes is the density of the lymph duct supply both to the stomach and to the lower esophagus, such that the cancers at the EGJ invade the regional lymph nodes concerned at an early stage[24].

Previous studies have proved that the number of lymph nodes retrieved has a significantly impact in pN category, which resulted in a "stage-migration" phenomenon[25-27]; therefore, in the present study the quality of lymphadenectomy was adequate because the median number of resected lymph nodes was clearly more than 15, as recommended by the Japanese Classification of Gastric Carcinoma: 3rd English edition[14]. Furthermore, the median number of 23 lymph nodes in our study is comparable with other prospective studies on the treatment of GC[27,28]. The number of dissected lymph nodes is closely associated with the pathological stages and prognosis. A population-based study by Bouvier *et al*[29] showed that the error rate was 47.1% if the pathological stages were classified according to the identical TNM stages for the patients with < 10 or > 15 detected lymph nodes. Thus, the pathological stages are not reliable for patients with < 10 detected lymph nodes in GC surgery. On TNM stages, Union for International Cancer Control version 5 states that the number of dissected lymph nodes in advanced GC must be ⩾ 15 to ensure the reliability of pathological stages and prognosis judgment. In a study reported by Karpeh *et al*[30], 27 patients with GC classified as stage Ⅱ and Ⅲ disease, and having < 15 lymph nodes examined, had significantly lower 5-year survival rates than those who had ⩾ 15 lymph nodes examined. In a similar analysis, Bouvier *et al*[29] concluded that > 10 lymph nodes should be analyzed per specimen to allow for valid N staging.

The sex distribution in this study showed an absolute male predominance (3.2:1) in gastric cardiac adenocarcinoma, which is similar to previous studies[23,31,32]. The sex ratio for cancer of the pylorus is only 1.5[33]. Although the exact reason for the male predominance of this type of cancer remains unknown, it seems to be a definite feature of this type of tumor, irrespective of the origin of the population[7,34]. Some scholars pointed out that this

may be because sex hormones such as estrogen affect the incidence rate of GC[35,36]. In our group, 98.6% of patients had tumors > 2 cm. Larger tumors have higher rates of lymph node metastases. Of the 104 cases with lymph node metastases, all the tumor sizes were > 2 cm, accounting for all metastases. Morphological classification was mainly of the ulcerative type (71.9%). Otherwise, type 0, 1, 3 and 4 accounted for 4.1%, 8.9%, 8.9% and 5.5%, respectively. Histologically, there were slightly more undifferentiated tumors (52.1%) than differentiated tumors (47.9%), and > 83.6% of patients had T3 or T4 tumors.

Toward the latter, the seventh edition of the TNM classification of malignant tumors defines rules for classifying carcinomas arising within the vicinity of the EGJ to end the imprecise regulation of earlier editions, where carcinomas around the EGJ could be staged according to either the classification of esophageal carcinomas or the classification of gastric carcinomas. However, neither of the two staging systems has proven to be clearly superior to the other, and neither of them is perfect for so-called cardiac adenocarcinomas. For the N classification of the so-called cadiac adenocarcinomas, both schemes are monotone and distinct, with continuously decreasing and significantly different prognosis with an increasing number of lymph node metastases[37]. Huang[38] pointed out that the Version 7 manual would predict the prognosis of patients more effectively than the Version 6 manual according to the staging of GC. The staging of lymph nodes (pN) can predict the prognosis better than the invasion depth of cancer tissue (pT), while the lymph node status in the axial area of the celiac artery is particularly critical. The Version 7 manual defined the EGJ-involved gastric cardia cancer staging improperly and this should be corrected. Of course, their research results are to be updated and verified with more large-sample studies. Huang *et al*[39] postulated that type Ⅱ EGJ adenocarcinomas are more adequately staged as GC by the seventh edition of the American Joint Committee on Cancer classification.

Many researchers have attempted to investigate the relationship between nodal involvement and clinicopathological factors. The factors related to lymph node metastasis include age, sex, clinical staging of tumor, pathological tissue type, invasion depth of lesion, tumor size, and typing. As expected, we found tumor characteristics such as tumor size, gross appearance, differentiation, pathological depth and lymphatic invasion were associated with lymph node metastases in all patients and male patients, and could represent a selection indicator of lymph node dissection. However, there was no obvious correlation between lymph node metastases and clinicopathological features in female patients. In gastric cardiac adenocarcinoma, the clinicopathological features and lymph node metastasis patterns did not differ significantly between male and female patients. These results were similar to those reported by previous studies[40]. Male patients had lymph node metastasis in 72.1%; slightly higher than that in female patients. The present study discovered that the metastasis rate of lymph nodes increased with the maximum diameter of the lesion; nevertheless, it is not advisable to simply take the tumor size as the correlation factor for predicting the lymph node metastasis because of variations in the period of tumor growth. Borrmann typing is also related to lymph node metastasis. The metastasis rate of lymph nodes in type Ⅲ and Ⅳ GC was significantly higher than in type Ⅰ and Ⅱ in this paper. This could be explained by the main invasion growth of the former types and the limited growth of the later types, because weak or strong invasion ability may lead to differences in the metastasis rate of lymph nodes. Histological type is closely related to nodal status. In our group, the rate of lymph node metastases in undifferentiated tumors was higher than that observed in differentiated cancer: 85.7% (60/70) and 67.9% (44/76), respectively. The tumor differentiation extent decides the biological behavior of GC. A larger extent of cell differentiation possibly causes a larger metastasis rate of lymph nodes. Some scholars have found that poorly differentiated GC cells produced more type Ⅳ collagenase, which can degrade the basilar membrane, reduce the ability to resist cancer cell infiltration, and cause the rate of lymph node metastasis to be higher than that for differentiated adenocarcinoma. Moreover, there is an increasing rate of node involvement as the T stage increases; in our series, 12.5% of T1, 50.0% of T2, 75.0% of T3 and 78.0% of T4 cases had positive nodes. This suggests a correlation between T stage factor and the presence of positive nodes. The results of this study showed that lymphatic duct invasion is closely related to the lymph node metastasis; the metastasis rate of lymph nodes was up to 100% in the LVI (+) group, but 0% in the LVI (-) groups. Many studies have shown that metastasis of lymphatic duct invasion occurs before lymph node metastasis. The presence of lymphatic duct invasion or cancer cells indicates the prophase of lymph node metastasis or a manifestation of lymph node metastasis. The above factors should be the focus of preoperative gastric cardia treatment options. The appropriate degree of lymph node dissection must selected to improve the surgical efficacy in gastric cardia cancer.

In this study, multivariate analysis revealed that tumor differentiation was the only independent risk factor for lymph node metastases in all patients, and revealed that tumor differentiation and pathological depth were independent risk factors for lymph node metastases in male patients. By logistic methods, Liu *et al*[41] also confirmed that the tumor length, invasion depth, blood vessel invasion and specimen stump had a significant effect on lymph node metastasis. With the increase of tumor length and invasion depth, the appearance of blood vessel invasion and specimen stump cancer cells, the risk of lymph node metastasis increased significantly.

The new nodal staging in the 7th TNM classification is based on the number of metastatic nodes. In our group, all 146 cases of gastric cardiac adenocarcinoma

received radical gastrectomy. Postoperatively, 3340 regional lymph nodes were located. Seven hundred and fifty-four lymph nodes were found in 104 cases with lymph node metastases - an average of 7.25 per case. It had been considered that all the regional nodes of the stomach were potentially involved in metastasis in patients with adenocarcinoma of the gastric cardia[42]. Lymphogenous metastasis by cancer of the cardia frequently affects the lymph nodes at the greater and lesser curvature of the stomach. Less frequent involvement of the lymph nodes at right cardial and left cardial lymph nodes has been observed[39,43,44]. In line with previous findings[12,45,46], the Mine *et al*[47] confirmed that nodal station numbers 3 (lesser curvature), 1 (right cardia), 2 (left cardia) and 7 (left gastric artery) were most frequently involved in type Ⅱ junctional cancers. The study of Hosokawa *et al*[40] came to a similar conclusion. The present study discovered that the perigastric lymph nodes (in Groups 3, 1, 4 and 2) in patients with the cardia cancer ranked the top four positions by metastasis rate, suggesting that the cardiac lymph node is a key dissection object in the reasonable radical operation.

Even after a precise anatomical-topographical differentiation of this tumor entity, Siewert *et al*[20] found a small number of patients with parapyloric node metastasis in their cohort with type Ⅱ adenocarcinoma. Consistent with their finding, in our patient series we found 4.1% of patients with suprapyloric node metastasis and 3.4% with infrapyloric node metastasis. Wang *et al*[48] reported that the pathological examination after total gastrectomy showed metastasis rates of lymph nodes in No. 5 and No. 6 of 9.1%-13.6%. They believed that it was difficult to remove all tumor tissues (including metastatic lymph nodes) without total gastrectomy.

Yamashita *et al*[34] clearly indicated that dissection of the paracardial and lesser curve lymph nodes offered significant therapeutic benefit, suggesting that these lymph nodes were possibly peritumoral. Furthermore, the number of metastatic nodes in these stations and the total number of metastatic nodes in all stations were equally predictive of the clinical outcome. Dissection of other perigastric nodes, such as Nos. 4sb, 4d, 5, and 6, offered only marginal therapeutic benefit as determined by calculating the index of estimated benefit of nodal dissection. Thus, involvement of the lymph nodes in these stations appeared to represent distant rather than locoregional metastasis[34]. Therefore, both esophagectomy with gastric tube reconstruction and gastrectomy with Roux-en-Y reconstruction seem to be valid procedures clinically.

Most series report 7%-40% of mediastinal nodal involvement for type Ⅱ and Ⅲ esophagogastric cancer even though abdominal nodes are more affected[49]. In our series, mediastinal lymph nodes were affected only in a small number (No. 110, 0.7%), lower than that reported in the literature[40,49,50]. The necessity of a prophylactic mediastinal nodal dissection remains controversial. Mine *et al*[47] suggested that lower mediastinal lymph nodes, and station numbers 16A2lat (left renal vein), 11 (splenic artery) and 9 (celiac axis) were the second most frequently involved, and positivity here influenced survival. Hiroharu's data[34] suggested that extensive mediastinal lymph node dissection *via* thoracotomy offers no survival benefit over para-periesophageal node clearance alone by the transhiatal approach, which is associated with a lower morbidity, consistent with Sasako' and Hulscher' finding[51,52]. Phase Ⅲ trials in The Netherlands (Dutch trial) and Japan (JCOG 9502) also suggested that an extended transthoracic resection was more hazardous surgery, in terms of morbidity, than a transhiatal esophagectomy. Extended surgery could not be recommended for patients with type Ⅱ tumors[52]. In addition, nodal recurrence was the most frequent in the para-aortic nodes, and less frequent in the mediastinal nodes in Hiroharu's series[34]. These results mostly consistent with another report[40] support the hypothesis that complete mediastinal nodal clearance is not essential for local control of this disease. Nevertheless, Reeh *et al*[53] showed that the presence of lower mediastinal lymph nodes in AOG(oesophagogastric junction) type Ⅱ suggests that at least a lower mediastinal dissection should be performed.

Lymph node Nos. 10 and 11 (splenic hilum and splenic artery) belong to pN_2 cardia cancer and a have a higher metastatic rate. The high risk factors include female sex, Borrmann type Ⅳ, tumor size > 5 cm, poorly differentiated adenocarcinoma, signet ring cell carcinoma, Lauren's diffuse type, vascular lymphatic invasion, and perineural invasion. Some authors believe that a splenectomy must be included for patients with the above high-risk factors[54]. Okajima *et al*[55] reported that the metastasis rates for lymph node Nos. 10 and 11 in cardia cancer were 15.5% and 12.1%, respectively, and Sakaguchi *et al*[56] estimated the rate at 24%. This reflects the status of lymph node metastasis; however, these data were derived from the pathological examination of surgical specimens, mostly based on the corresponding radical operation, and was subject to the understanding of radical surgical indications. Sakaguchi *et al*[56] believed that the lymph node metastasis of Nos. 10 and 11 in a larger tumor (> 4 cm), with deeper lesions (T3 and T4) and infiltrative lesions occurred easily. Thus, these clinical characteristics may provide a reference for understanding the indications for combined splenectomy. In our study, the metastasis rates for lymph node No. 10 lymph 1.4%, and for No. 11 it was 2.7%, which are lower than those reported in the literature[39,40,57]. Metastasis in these lymph nodes was mainly observed in advanced GC. Therefore, it would be prudent to select the combined resection of distal pancreatectomy and splenectomy for lymph node dissection in patients with cardia cancer[58,59].

The present study showed that the most common sites of the pN_1 lymph node metastasis were Nos. 1-4; the most common sites of the pN_2 lymph nodes were Nos. 7-9; and the most common sites of the pN_3 lymph nodes were Nos. 5 and 6. Lymph node metastasis occurred mostly in the abdominal cavity and lymph node

metastasis of cardia cancer is more similar to that seen for GC. The lymph node metastasis in cardia cancer observed in this study suggests that: (1) for lymph nodes Nos. 1-9, conditions must be focally examined in preoperative ultrasound endoscopic and computed tomography examination; and (2) the superior paragastric fatty tissues should be thoroughly removed in the radical operation for GC and the total lymph node should be dissected in the regions; the celiac trunk and common hepatic artery must be skeletonized and the left gastric artery must be cut to remove the Nos. 7-9 lymph nodes thoroughly.

One analysis showed that 32.9% of type Ⅱ tumors had involvement of the lymph nodes along the major branched arteries (the left gastric artery, common hepatic artery, splenic artery and celiac axis), and the rate was 50% in type Ⅲ tumors[60]. Siewert *et al*[61] also reported similar results; 25% nodal involvement in type Ⅱ tumors and 39% in type Ⅲ tumors. These reports clearly indicate that abdominal nodal metastases are frequently observed in adenocarcinoma of the esophagogastric junction type Ⅱ/Ⅲ tumors, as in true gastric cancer**.** Therefore, the extent of a nodal dissection for AEG type Ⅱ/Ⅲ should be same as that applied for GC, and an abdominal D2 lymphadenectomy is recommended for patients with type Ⅱ/Ⅲ tumors, unless D2 increases the surgical risk[32]. Siewert types Ⅱ and Ⅲ cancers could be removed safely with an abdominal approach[45]. Our results agree with the conclusion of Husemann, that carcinoma of the cardia is a type of carcinoma of the stomach that must be treated according to the criteria of GC surgery[50].

In our study, of 104 patients with lymph node metastases, all were N1, 23 were N2, and 15 were N3. Investigating the correlation between pN_1, pN_2 and pN_3 lymph node metastases and clinicopathological factors, we found that tumor size, gross appearance, differentiation, pathological depth and lymphatic invasion were associated with lymph node metastases in all patients and male patients at pN_1. There was an obvious correlation between lymph node metastases and lymphatic invasion in all patients at pN_2. Univariate analysis of variance revealed a close relationship between lymph node metastases and lymphatic invasion and neural invasion in all patients and lymphatic invasion in female patients at pN_3. Study of Di Leo *et al*[10] study of the treatment of advanced gastric cancer showed that T2 tumors were consistently associated with pN_2 stations nodal infiltration. Such behavior, although less frequent than in T3/4 tumors, does not allow conservative surgery in terms of nodal resection[10].

There were limitations to the present study. First, it was a retrospective study based on postoperative examination of resected specimens. Second, the number of patients was low. Thus, further study with a larger sample size should be carried out to confirm our results. Otherwise, the extent of nodal involvement was most likely underestimated. The lack of information of nodal status at specific remote sites in some cases also made the investigation of nodal stage migration impossible. The retrospective nature of this study meant that there was some selection bias, such as the surgeon's preference for a thoracoabdominal or transabdominal approach.

In conclusion, the findings in this study indicate that the clinicopathological features and risk factors for lymph node metastasis of male and female patients with gastric cardiac adenocarcinoma did not differ significantly. Therefore, the effect of male sex on the clinical course of gastric cardiac adenocarcinoma had a weak impact in comparison to female sex once a curative resection had been performed. However, further evaluations should be performed. The outcome should improve if male patients, as well as female patients, undergo careful diagnosis of malignancy and early multimodality treatment**.**

COMMENTS

Background

As gastric cancer incidence declines, the frequency of proximal gastric and gastroesophageal junctional adenocarcinomas continues to rise, and has become a significant clinical challenge. Adenocarcinoma of the cardia generally has a low curative resection rate and a poor prognosis; worse than carcinoma of other regions of the stomach, mainly because the disease is at a more advanced stage at diagnosis. It is crucial that the therapeutic strategy for gastric cardiac adenocarcinoma be clarified through evaluation of both the pattern of lymph node metastasis and the efficacy of lymph node dissection in this region.

Research frontiers

The optimal surgical strategy for tumors in the cardiac area of the stomach, especially tumors invading the lower esophagus, remains controversial. The development of effective therapeutic strategies for these tumors requires information on patient characteristics, patterns of lymph node metastasis, and the efficacy of lymph node dissection.

Innovations and breakthroughs

Univariate analysis showed an obvious correlation between lymph node metastases and tumor size, gross appearance, differentiation, pathological depth and lymphatic invasion in male patients. Multivariate logistic regression analysis revealed that tumor differentiation and pathological depth were independent risk factors for lymph node metastases in male patients. There was an obvious relationship between lymph node metastases and tumor size, gross appearance, differentiation, pathological depth, lymphatic invasion at pN_1, and lymphatic invasion at pN_2 and neural invasion at pN_3 in male patients. There were no significant differences in clinicopathological features or lymph node metastases between female and male patients.

Applications

Tumor differentiation and depth were risk factors for lymph node metastases in male patients with gastric cardiac adenocarcinoma and should be considered when choosing surgery.

Terminology

The definition of the cardia commonly employed in Japan is the area within 2 cm above and below the esophagogastric junction, and tumors whose center is situated in this area are considered to be cancer of the cardia; such cancers are distinguished from upper gastric cancers. Siewert and Stein proposed a topographical classification for cardiac carcinomas.

Peer review

Congratulate the authors for an excellent effort. As rightly highlighted, a future attempt in expanding the population size should hopefully provide further insights.

REFERENCES

1 **Brenner H**, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. *Methods Mol Biol* 2009; **472**: 467-477 [PMID: 19107449 DOI: 10.1007/978-1-60327-492-0_23]

2 **Kamangar F**, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents:

defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 2006; **24**: 2137-2150 [PMID: 16682732 DOI: 10.1200/JCO.2005.05.2308]
3 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]
4 **Zou XN**, Sun XB, Chen WQ, Zheng RS, Zhang SW, Dai Z, Liu WD. Analysis of incidence and mortality of gastric cancer in China in 2003-2007. *Zhongliu* 2012; **32**: 109-114
5 **Devesa SS**, Blot WJ, Fraumeni JF. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998; **83**: 2049-2053 [PMID: 9827707]
6 **Kunisaki C**, Shimada H, Nomura M, Matsuda G, Otsuka Y, Ono H, Akiyama H. Surgical outcome in patients with gastric adenocarcinoma in the upper third of the stomach. *Surgery* 2005; **137**: 165-171 [PMID: 15674196 DOI: 10.1016/j.surg.2004.06.005]
7 **Bruno L**, Nesi G, Montinaro F, Carassale G, Lassig R, Boddi V, Bechi P, Cortesini C. Clinicopathologic findings and results of surgical treatment in cardiac adenocarcinoma. *J Surg Oncol* 2000; **74**: 33-35 [PMID: 10861606]
8 **Fontana MG**, La Pinta M, Moneghini D, Villanacci V, Donato F, Rindi G, Paparini S, Baronchelli C, Bertoli G, Alquati P. Prognostic value of Goseki histological classification in adenocarcinoma of the cardia. *Br J Cancer* 2003; **88**: 401-405 [PMID: 12569383 DOI: 10.1038/sj.bjc.6600663]
9 **Graham AJ**, Finley RJ, Clifton JC, Evans KG, Fradet G. Surgical management of adenocarcinoma of the cardia. *Am J Surg* 1998; **175**: 418-421 [PMID: 9600291]
10 **Di Leo A**, Marrelli D, Roviello F, Bernini M, Minicozzi A, Giacopuzzi S, Pedrazzani C, Baiocchi LG, de Manzoni G. Lymph node involvement in gastric cancer for different tumor sites and T stage: Italian Research Group for Gastric Cancer (IRGGC) experience. *J Gastrointest Surg* 2007; **11**: 1146-1153 [PMID: 17576611]
11 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma - 2nd English Edition - *Gastric Cancer* 1998; **1**: 10-24 [PMID: 11957040 DOI: 10.1007/s101200100009]
12 **Rüdiger Siewert J**, Feith M, Werner M, Stein HJ. Adenocarcinoma of the esophagogastric junction: results of surgical therapy based on anatomical/topographic classification in 1,002 consecutive patients. *Ann Surg* 2000; **232**: 353-361 [PMID: 10973385]
13 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011; **14**: 113-123 [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]
14 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; **14**: 101-112 [PMID: 21573743 DOI: 10.1007/s10120-011-0041-5]
15 **Ichikura T**, Ogawa T, Kawabata T, Chochi K, Sugasawa H, Mochizuki H. Is adenocarcinoma of the gastric cardia a distinct entity independent of subcardial carcinoma? *World J Surg* 2003; **27**: 334-338 [PMID: 12607062 DOI: 10.1007/s00268-002-6776-8]
16 **Kunisaki C**, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono H, Shimada H. Surgical outcomes for early gastric cancer in the upper third of the stomach. *J Am Coll Surg* 2005; **200**: 15-19 [PMID: 15631915 DOI: 10.1016/j.jamcollsurg.2004.09.015]
17 **Saito H**, Fukumoto Y, Osaki T, Fukuda K, Tatebe S, Tsujitani S, Ikeguchi M. Distinct recurrence pattern and outcome of adenocarcinoma of the gastric cardia in comparison with carcinoma of other regions of the stomach. *World J Surg* 2006; **30**: 1864-1869 [PMID: 16983479 DOI: 10.1007/s00268-005-0582-z]
18 **Wijetunge S**, Ma Y, DeMeester S, Hagen J, DeMeester T, Chandrasoma P. Association of adenocarcinomas of the distal esophagus, "gastroesophageal junction," and "gastric cardia" with gastric pathology. *Am J Surg Pathol* 2010; **34**: 1521-1527 [PMID: 20871225 DOI: 10.1097/PAS.0b013e3181eff133]
19 **Kong SH**, Yoo MW, Kim JW, Lee HJ, Kim WH, Lee KU, Yang HK. Validation of limited lymphadenectomy for lower-third gastric cancer based on depth of tumour invasion. *Br J Surg* 2011; **98**: 65-72 [PMID: 20954197 DOI: 10.1002/bjs.7266]
20 **Siewert JR**, Stein HJ. Classification of adenocarcinoma of the oesophagogastric junction. *Br J Surg* 1998; **85**: 1457-1459 [PMID: 9823902]
21 **Mariette C**, Castel B, Toursel H, Fabre S, Balon JM, Triboulet JP. Surgical management of and long-term survival after adenocarcinoma of the cardia. *Br J Surg* 2002; **89**: 1156-1163 [PMID: 12190682 DOI: 10.1046/j.1365-2168.2002.02185.x]
22 **de Manzoni G**, Pedrazzani C, Pasini F, Di Leo A, Durante E, Castaldini G, Cordiano C. Results of surgical treatment of adenocarcinoma of the gastric cardia. *Ann Thorac Surg* 2002; **73**: 1035-1040 [PMID: 11996237 DOI: 10.1016/S0003-4975(01)03571-8]
23 **Hamabe Y**, Ikuta H, Nakamura Y, Kawasaki K, Yamamoto M. Clinicopathological features of esophageal cancer simultaneously associated with gastric cancer. *J Surg Oncol* 1998; **68**: 179-182 [PMID: 9701211]
24 **Casson AG**, Darnton SJ, Subramanian S, Hiller L. What is the optimal distal resection margin for esophageal carcinoma? *Ann Thorac Surg* 2000; **69**: 205-209 [PMID: 10654514 DOI: 10.1016/S0003-4975(99)01262-X]
25 **de Manzoni G**, Verlato G, Roviello F, Morgagni P, Di Leo A, Saragoni L, Marrelli D, Kurihara H, Pasini F. The new TNM classification of lymph node metastasis minimises stage migration problems in gastric cancer patients. *Br J Cancer* 2002; **87**: 171-174 [PMID: 12107838 DOI: 10.1038/sj.bjc.6600432]
26 **Wu CW**, Hsiung CA, Lo SS, Hsieh MC, Chen JH, Li AF, Lui WY, Whang-Peng J. Stage migration influences on stage-specific survival comparison between D1 and D3 gastric cancer surgeries. *Eur J Surg Oncol* 2005; **31**: 153-157 [PMID: 15698731]
27 **Barbour AP**, Rizk NP, Gonen M, Tang L, Bains MS, Rusch VW, Coit DG, Brennan MF. Lymphadenectomy for adenocarcinoma of the gastroesophageal junction (GEJ): impact of adequate staging on outcome. *Ann Surg Oncol* 2007; **14**: 306-316 [PMID: 17091329 DOI: 10.1245/s10434-006-9166-x]
28 **Hölscher AH**, Drebber U, Mönig SP, Schulte C, Vallböhmer D, Bollschweiler E. Early gastric cancer: lymph node metastasis starts with deep mucosal infiltration. *Ann Surg* 2009; **250**: 791-797 [PMID: 19809298 DOI: 10.1097/SLA.0b013e3181bdd3e4]
29 **Bouvier AM**, Haas O, Piard F, Roignot P, Bonithon-Kopp C, Faivre J. How many nodes must be examined to accurately stage gastric carcinomas? Results from a population based study. *Cancer* 2002; **94**: 2862-2866 [PMID: 12115373 DOI: 10.1002/cncr.10550]
30 **Karpeh MS**, Leon L, Klimstra D, Brennan MF. Lymph node staging in gastric cancer: is location more important than Number? An analysis of 1,038 patients. *Ann Surg* 2000; **232**: 362-371 [PMID: 10973386 DOI: 10.1097/00000658-200009000-00008]
31 **Yoon HY**, Kim CB. Gastroesophageal junction adenocarcinoma of young patients who underwent curative surgery: a comparative analysis with older group. *Surg Today* 2011; **41**: 203-209 [PMID: 21264755 DOI: 10.1007/s00595-009-4228-x]
32 **Hasegawa S**, Yoshikawa T. Adenocarcinoma of the esophagogastric junction: incidence, characteristics, and treatment strategies. *Gastric Cancer* 2010; **13**: 63-73 [PMID: 20602191 DOI: 10.1007/s10120-010-0555-2]
33 **Sterup K**, Mosbech J. Sex ratio of gastric cancer related to site of the tumour. *Scand J Gastroenterol Suppl* 1971; **9**: 87-89 [PMID: 4327215]
34 **Yamashita H**, Katai H, Morita S, Saka M, Taniguchi H, Fukagawa T. Optimal extent of lymph node dissection for

Siewert type II esophagogastric junction carcinoma. *Ann Surg* 2011; **254**: 274-280 [PMID: 21772128 DOI: 10.1097/SLA.0b013e3182263911]
35 **Harrison JD**, Jones JA, Ellis IO, Morris DL. Oestrogen receptor D5 antibody is an independent negative prognostic factor in gastric cancer. *Br J Surg* 1991; **78**: 334-336 [PMID: 2021850 DOI: 10.1002/bjs.1800780321]
36 **La Vecchia C**, D'Avanzo B, Franceschi S, Negri E, Parazzini F, Decarli A. Menstrual and reproductive factors and gastric-cancer risk in women. *Int J Cancer* 1994; **59**: 761-764 [PMID: 7989115 DOI: 10.1002/ijc.2910590609]
37 **Gertler R**, Stein HJ, Loos M, Langer R, Friess H, Feith M. How to classify adenocarcinomas of the esophagogastric junction: as esophageal or gastric cancer? *Am J Surg Pathol* 2011; **35**: 1512-1522 [PMID: 21934477 DOI: 10.1097/PAS.0b013e3182294764]
38 **Huang Q**. Definition of Gastric-esophageal junctional area and progress of tumor classification and staging. *Zhonghua Xiaohua Neijing Zazhi* 2011; **28**: 121
39 **Huang Q**, Shi J, Feng A, Fan X, Zhang L, Mashimo H, Cohen D, Lauwers G. Gastric cardiac carcinomas involving the esophagus are more adequately staged as gastric cancers by the 7th edition of the American Joint Commission on Cancer Staging System. *Mod Pathol* 2011; **24**: 138-146 [PMID: 20852593 DOI: 10.1038/modpathol.2010.183]
40 **Wayman J**, Bennett MK, Raimes SA, Griffin SM. The pattern of recurrence of adenocarcinoma of the oesophago-gastric junction. *Br J Cancer* 2002; **86**: 1223-1229 [PMID: 11953876 DOI: 10.1038/sj.bjc.6600252]
41 **Liu W**, Hao XS, Chen Y, Li HX, Wang SJ, Wang PZ, Fan Q, Jin Y, Guan LY, Song LN, Ping YM, Meng XL, Wang R, Liu JF, Wang XL. Analysis of lymph node metastasis in 1526 cases with thoracic esophageal cancer and cardia cancer (attached with random sampling report of the Fourth Hospital of Hebei Medical University from 1996 to 2004). *Zhongguo Zhongliu Linchuang* 2008; **35**: 601-605
42 **Papachristou DN**, Fortner JG. Adenocarcinoma of the gastric cardia. The choice of gastrectomy. *Ann Surg* 1980; **192**: 58-64 [PMID: 7406565 DOI: 10.1097/00000658-198007000-00010]
43 **Sons HU**, Borchard F. Cancer of the distal esophagus and cardia. Incidence, tumorous infiltration, and metastatic spread. *Ann Surg* 1986; **203**: 188-195 [PMID: 3947155 DOI: 10.1097/00000658-198602000-00013]
44 **Ielpo B**, Pernaute AS, Elia S, Buonomo OC, Valladares LD, Aguirre EP, Petrella G, Garcia AT. Impact of number and site of lymph node invasion on survival of adenocarcinoma of esophagogastric junction. *Interact Cardiovasc Thorac Surg* 2010; **10**: 704-708 [PMID: 20154347 DOI: 10.1510/icvts.2009.222778]
45 **Fang WL**, Wu CW, Chen JH, Lo SS, Hsieh MC, Shen KH, Hsu WH, Li AF, Lui WY. Esophagogastric junction adenocarcinoma according to Siewert classification in Taiwan. *Ann Surg Oncol* 2009; **16**: 3237-3244 [PMID: 19636628 DOI: 10.1245/s10434-009-0636-9]
46 **Pedrazzani C**, de Manzoni G, Marrelli D, Giacopuzzi S, Corso G, Minicozzi AM, Rampone B, Roviello F. Lymph node involvement in advanced gastroesophageal junction adenocarcinoma. *J Thorac Cardiovasc Surg* 2007; **134**: 378-385 [PMID: 17662776 DOI: 10.1016/j.jtcvs.2007.03.034]
47 **Mine S**, Sano T, Hiki N, Yamada K, Nunobe S, Yamaguchi T. Lymphadenectomy around the left renal vein in Siewert type II adenocarcinoma of the oesophagogastric junction. *Br J Surg* 2013; **100**: 261-266 [PMID: 23180514 DOI: 10.1002/bjs.8967]
48 **Wang LS**, Wu CW, Hsieh MJ, Fahn HJ, Huang MH, Chien KY. Lymph node metastasis in patients with adenocarcinoma of gastric cardia. *Cancer* 1993; **71**: 1948-1953 [PMID: 8443744]
49 **Dresner SM**, Lamb PJ, Bennett MK, Hayes N, Griffin SM. The pattern of metastatic lymph node dissemination from adenocarcinoma of the esophagogastric junction. *Surgery* 2001; **129**: 103-109 [PMID: 11150040 DOI: 10.1067/msy.2001.110024]
50 **Husemann B**. Cardia carcinoma considered as a distinct clinical entity. *Br J Surg* 1989; **76**: 136-139 [PMID: 2702444 DOI: 10.1002/bjs.1800760210]
51 **Sasako M**, Sano T, Yamamoto S, Sairenji M, Arai K, Kinoshita T, Nashimoto A, Hiratsuka M. Left thoracoabdominal approach versus abdominal-transhiatal approach for gastric cancer of the cardia or subcardia: a randomised controlled trial. *Lancet Oncol* 2006; **7**: 644-651 [PMID: 16887481 DOI: 10.1016/S1470-2045(06)70766-5]
52 **Hulscher JB**, van Sandick JW, de Boer AG, Wijnhoven BP, Tijssen JG, Fockens P, Stalmeier PF, ten Kate FJ, van Dekken H, Obertop H, Tilanus HW, van Lanschot JJ. Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the esophagus. *N Engl J Med* 2002; **347**: 1662-1669 [PMID: 12444180 DOI: 10.1056/NEJMoa022343]
53 **Reeh M**, Mina S, Bockhorn M, Kutup A, Nentwich MF, Marx A, Sauter G, Rösch T, Izbicki JR, Bogoevski D. Staging and outcome depending on surgical treatment in adenocarcinomas of the oesophagogastric junction. *Br J Surg* 2012; **99**: 1406-1414 [PMID: 22961520 DOI: 10.1002/bjs.8884]
54 **Shin SH**, Jung H, Choi SH, An JY, Choi MG, Noh JH, Sohn TS, Bae JM, Kim S. Clinical significance of splenic hilar lymph node metastasis in proximal gastric cancer. *Ann Surg Oncol* 2009; **16**: 1304-1309 [PMID: 19241107 DOI: 10.1245/s10434-009-0389-5]
55 **Okajima K**, Isozaki H. Splenectomy for treatment of gastric cancer: Japanese experience. *World J Surg* 1995; **19**: 537-540 [PMID: 7676696 DOI: 10.1007/BF00294715]
56 **Sakaguchi T**, Sawada H, Yamada Y, Fujimoto H, Emoto K, Takayama T, Ueno M, Nakajima Y. Indication of splenectomy for gastric carcinoma involving the proximal part of the stomach. *Hepatogastroenterology* 2001; **48**: 603-605 [PMID: 11379363]
57 **de Manzoni G**, Verlato G, Guglielmi A, Laterza E, Tomezzoli A, Pelosi G, Di Leo A, Cordiano C. Classification of lymph node metastases from carcinoma of the stomach: comparison of the old (1987) and new (1997) TNM systems. *World J Surg* 1999; **23**: 664-669 [PMID: 10390583 DOI: 10.1007/PL00012365]
58 **Kunisaki C**, Makino H, Suwa H, Sato T, Oshima T, Nagano Y, Fujii S, Akiyama H, Nomura M, Otsuka Y, Ono HA, Kosaka T, Takagawa R, Ichikawa Y, Shimada H. Impact of splenectomy in patients with gastric adenocarcinoma of the cardia. *J Gastrointest Surg* 2007; **11**: 1039-1044 [PMID: 17514409 DOI: 10.1007/s11605-007-0186-z]
59 **Holdsworth RJ**, Irving AD, Cuschieri A. Postsplenectomy sepsis and its mortality rate: actual versus perceived risks. *Br J Surg* 1991; **78**: 1031-1038 [PMID: 1933181]
60 **Hasegawa S**, Yoshikawa T, Cho H, Tsuburaya A, Kobayashi O. Is adenocarcinoma of the esophagogastric junction different between Japan and western countries? The incidence and clinicopathological features at a Japanese high-volume cancer center. *World J Surg* 2009; **33**: 95-103 [PMID: 18958523 DOI: 10.1007/s00268-008-9740-4]
61 **Siewert JR**, Stein HJ, Feith M. Adenocarcinoma of the esophago-gastric junction. *Scand J Surg* 2006; **95**: 260-269 [PMID: 17249275]

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BRIEF ARTICLE

Research on stress-induced apoptosis of natural killer cells and the alteration of their killing activity in mouse liver

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Abstract

AIM: To investigate the stress-induced apoptosis of natural killer (NK) cells and the changes in their killing activity in mouse livers.

METHODS: A restraint stress model was established in mice. Flow cytometry was employed to measure the percentage of NK cells and the changes in their absolute number in mouse liver. The cytotoxicity of hepatic and splenic NK cells was assessed against YAC-1 target cells *via* a 4 h 51Cr-release assay.

RESULTS: The restraint stress stimulation induced the apoptosis of NK cells in the liver and the spleen, which decreased the cell number. The number and percentage of NK cells in the spleen decreased. However, the number of NK cells in the liver decreased, whereas the percentage of NK cells was significantly increased. The apoptosis of NK cells increased gradually with prolonged stress time, and the macrophage-1 (Mac-1)$^+$ NK cells were more susceptible to apoptosis than Mac-1$^-$ NK cells. Large numbers of Mac-1$^-$ NK cells in the liver, which are more resistant to stress-induced apoptosis, were observed than the Mac-1$^-$ NK cells in the spleen. The stress stimulation diminished the killing activity of NK cells in the spleen was significantly decreased, but the retention of numerous Mac-1$^-$ NK cells in the liver maintained the killing ability.

CONCLUSION: Significant stress-induced apoptosis was observed among Mac-1$^+$ NK cells, but not Mac-1$^-$ NK cells in the mouse liver. Stress stimulation markedly decreased the killing activity of NK cells in the spleen but remained unchanged in the liver.

Key words: Restraint stress; Natural killer cells; Cell apoptosis; Killing activity

Core tip: Hepatic natural killer (NK) cells are classified into macrophage-1 (Mac-1)$^+$ and Mac-1$^-$ cells, and the different functional characteristics of Mac-1$^+$ or Mac-1$^-$ NK cells in response to stress stimulation are confirmed. This study further proves the heterogeneity of NK cell function, and the results provide a reference for preventing the immune system damage caused by stress.

Ma Z, Liu Y, Zhou X, Yu HL, Li MQ, Tomiyama-Miyaji C, Abo T, Bai XF. Research on stress-induced apoptosis of natural killer cells and the alteration of their killing activity in mouse liver. *World J Gastroenterol* 2013; 19(37): 6258-6264 Available from:

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INTRODUCTION

In the modern society, the acceleration of work and life style and the deterioration of the environment, as well as natural disasters and frequent traffic accidents, expose people to increasing stress. Prolonged or intense stress that overwhelms autoregulation, causes nervous, endocrine, and immune system dysfunction[1-3], as well as the apoptosis of lymphocytes such as natural killer (NK) cells, B cells and T cells. Immune system dysfunction is the direct cause of infectious diseases, cancers and self-deterioration[4-6].

NK cells are an important type of lymphocytes, accounting for 10% to 15% of total lymphocytes and play a crucial role in body resistance to against infections and tumours, as well as immune and hematopoietic regulation[7,8].

This study aims to determine the effects of persistent and intense stress on the apoptosis of hepatic and splenic NK cells and change in their function in mice.

MATERIALS AND METHODS

Experimental animals

Eight-week-old clean grade C57BL/6 mice were purchased from the Animal Centre of The 3rd Affiliated Hospital of Harbin Medical University.

Mouse model preparation

The protocol used has been described in the published literature[9]. The mice were placed in a 50 mL Falcon tube with 4 to 5 drilled holes at the bottom to maintain ventilation. Sufficient amounts of absorbent cotton were placed inside the tube to immobilise the mouse, and then the lid was screwed shut. The tube was kept at room temperature for 24 h, and the mouse was not fed any food and water. The mice in the control group were left in the original cage without any disturbance.

Mouse lymphocyte preparation

The protocol used has been described in the published literature[10]. The mouse was anaesthetised with ether and sacrificed *via* heart puncture. Subsequently, the mouse liver and spleen were collected and minced. The tissue sample was washed with phosphate-buffered saline (PBS) and filtered with 200 mesh strainer, and then the cell suspension was collected. After gradient centrifugation, the cells were lysed with 0.83% NH_4Cl-Tris buffer (pH 7.6). The resulting cell suspension was collected and the concentration was adjusted to 1.0×10^6/mL.

Immunofluorescence labelling

Lymphocytes were isolated from mouse liver and spleen, and then double or triple immunofluorescence staining was performed to identify the $CD_3^-NK_{1.1}^+$ cells as NK cells. Fluorescein-isothiocyanate (FITC)-labelled antibodies: CD_3 (145-2C11 clone); PE-labelled antibody: $NK_{1.1}$ (PK136 clone); Biotin-labelled antibody: macrophage-1 (Mac-1) (M1/70 clone) CD_{69} (H1.2F3 clone), Ly49C/I (5E6 clone). All the monoclonal antibodies were purchased from BD Biosciences Pharmingen in San Diego, United States.

Cell suspension was transferred in centrifuge tube (cell number < 2×10^6). After 2 min of centrifugation at 2500 r/min and 4 ℃, the supernatant was removed, followed by vibration. Then, 10 μL of 2.4 G2 was added (anti-FcγR Ⅱ/Ⅲ). After incubation at 4 ℃ for 10 min, 10 μL of various monoclonal antibodies (CD_3, $NK_{1.1}$, Mac-1, CD_{69}, and Ly49C) were added, accordingly. After vortex and incubation at 4 ℃ for 20 min, the cells were washed once with PBS (2500 r/min at 4 ℃). For double staining, the cells were diluted with 0.5 mL of PBS and filtered with nylon mesh, and then 5 μL of propidium iodide (PI) was added for flow cytometry analysis. When subjected for triple staining, cells were incubated with 10 μL of biotin-labelled secondary antibody at 4 ℃ for 20 min, and then washed with PBS once (2500 r/min at 4 ℃). After diluting with 0.5 mL of PBS and filtration with a nylon mesh, the stained cells were analyzed *via* flow cytometry[11]. Flow Cytometer was FAC sort from BD-United States and software was Cell Quest 3.0.

Detection of killing activity of NK cells

The cytotoxicity of NK cells was assessed against YAC-1 target cells. Target cells were continuously cultured for 24 h in RPMI 1640 containing 200 mL/L FCS. YAC-1 cells were collected at exponential phase and counted through trypan blue staining. Viable cells were considered as targets cells when their percentage exceeded 95%. The cell concentration was adjusted to 1×10^5/mL with RPMI 1640. After incubation with ^{51}Cr for 2 h, the cells were washed three times with RPMI 1640 to remove free ^{51}Cr. The target cell concentration was adjusted to 1.0×10^4/mL or 2.0×10^4/mL. The cells were divided into three groups: NK cell group, target cell maximum release group, and target cell spontaneous release group. Subsequently, the cells were seeded in U-bottom microplates (96-well). Lymphocytes in the mouse liver and spleen were utilized as effector cells, which were added into the U-bottom microplates at an effectoritarget ratio of 50:1, 25:1, and 12.5:1 in a volume of 100 μL/well. The cells were incubated at 37 ℃ with 5% CO_2 for 4 h and the microplates were centrifuged at 1500 r/min for 5 min. About 100 μL of supernatant was collected from each well, and its radioactivity (CPM) was measured with gamma counter[12].

The specific killing rate was calculated using the following formula: specific killing rate (%) = (experimental cell release - target cell spontaneous release)/(target cell maximum release - target cell spontaneous release) × 100%.

Statistical analysis

The data are presented as mean ± SD and percentage.

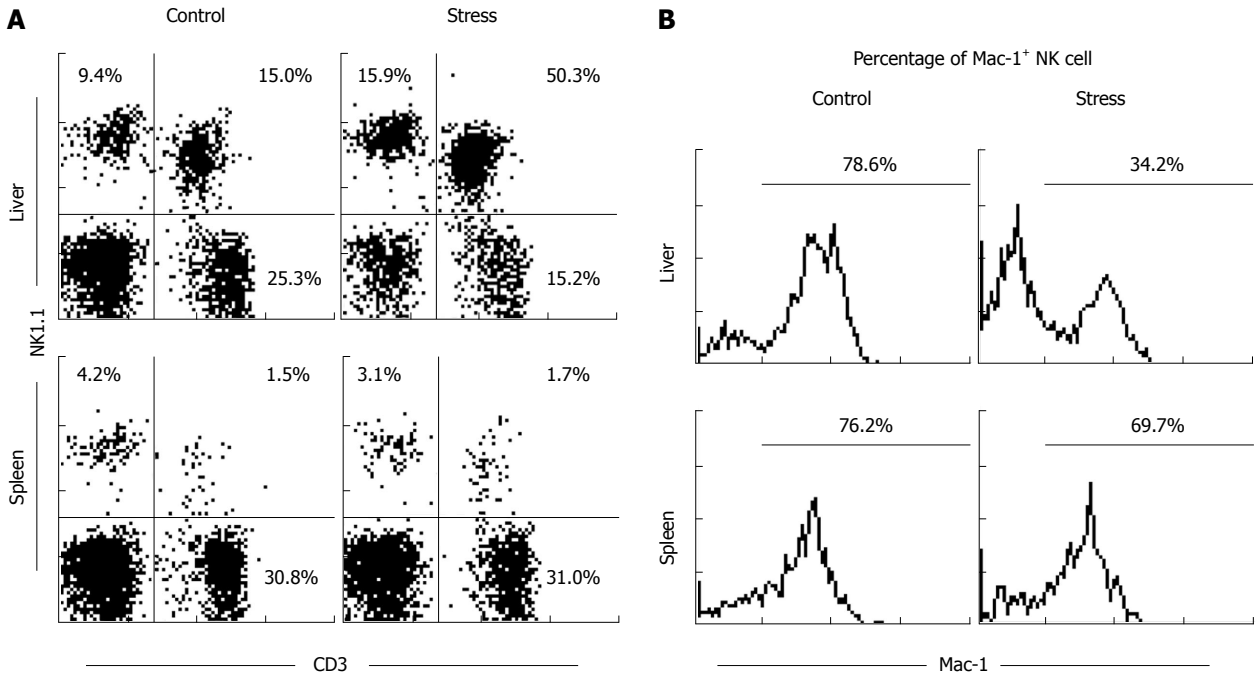

Figure 1 Percentage of natural killer cells and macrophage-1$^+$ natural killer cells in mouse liver and spleen after 24 h of restraint stress. A: Natural killer cells; B: Macrophage-1$^+$ natural killer cells. NK: Natural killer; Mac-1: Macrophage-1.

Significant differences between two samples were analyzed with Student's *t* test.

RESULTS

Stress stimulation and the change of NK cell number

The percentage of splenic NK cells in total lymphocytes did not change significantly (3.9% ± 1.2% *vs* 2.6% ± 1.1%, $P > 0.05$), whereas the percentage of hepatic NK cells increased (8.6% ± 1.3% *vs* 14.9% ± 1.5%, $P < 0.05$; Figure 1).

After restraint stress stimulation, the numbers of lymphocytes in the experimental and control groups were (53.1 ± 9.7) × 10^5 *vs* (19.7 ± 4.6) × 10^5 ($n = 6$, $P < 0.05$) in liver, (87.7 ± 9.6) × 10^6 *vs* (36.4 ± 7.1) × 10^6 ($n = 6$, $P < 0.05$) in spleen. The number of NK cells in the experimental and control groups were (57.4 ± 8.9)× 10^5 *vs* (24.6 ± 7.3)× 10^5 ($n = 6$, $P < 0.05$) in the liver, (29.7 ± 6.5) × 10^6 *vs* (8.6 ± 1.4)× 10^6 ($n = 6$, $P < 0.05$) in the spleen (Figure 2).

Number of Mac-1$^+$ and Mac-1$^-$ NK cells

NK cells were isolated from mouse liver and spleen after 24 h of restraint stress stimulation, followed by immunofluorescence staining with FITC: Mac-1 and PE: $NK_{1.1}$. The cells were analyzed *via* flow cytometry. The results show that the percentage of hepatic Mac-1$^+$ NK cells in the experimental group was significantly higher than that of the control group (77.2% ± 1.7% *vs* 33.9% ± 1.1%, $P < 0.05$, Figure 1B), whereas the percentage of hepatic Mac-1$^-$ NK cells relatively increased. The percentage of splenic Mac-1$^+$ NK cells was slightly decreased (75.1% ± 1.1% *vs* 68.5% ± 1.6%, $P > 0.05$).

After 24 h of stress stimulation, the number of Mac-1$^+$ NK cells in the liver and spleen in the stress group was significantly lower than those in the control group: liver, (37.7 ± 9.8) × 10^4 *vs* (8.4 ± 1.7) × 10^4 ($n = 6$, $P < 0.05$); spleen, (23.5 ± 6.3) × 10^5 *vs* (8.7 ± 1.9) × 10^5 ($n = 6$, $P < 0.05$). The results are shown in Figure 3.

Stress stimulation and apoptosis of NK cells

NK cells were isolated from mouse liver after 24 h of restraint stress stimulation, followed by immunofluorescence staining with Annexin V-FITC and PI. The cells were analyzed *via* flow cytometry. The results revealed significant apoptosis of NK cells (9.5% ± 1.4% *vs* 19.3% ± 1.3%, $P < 0.05$), especially the Mac-1$^+$ NK cells (5.2% ± 1.8% *vs* 19.3% ± 1.4%, $P < 0.05$), whereas the apoptosis of Mac-1$^-$ NK cells did not significantly change (13.4% ± 1.3% *vs* 7.4% ± 1.7%, $P > 0.05$; Figure 4).

Stress stimulation and the change of killing activity mediated by NK cells

The killing activity of NK cells was markedly decreased in the spleen (16.7% ± 1.4% *vs* 8.9% ± 1.1%, $P < 0.05$), but was sustained in the liver even after 24 h of restraint stress stimulation (Figure 5).

DISCUSSION

With the development of modern medical technology, increasing attention has been focused on the relationship between stress and health. The response of the body to stress is a dynamic balance, which allows the body to recover to its original state through autoregulation after stress reaction. However, persistent stress may overwhelm

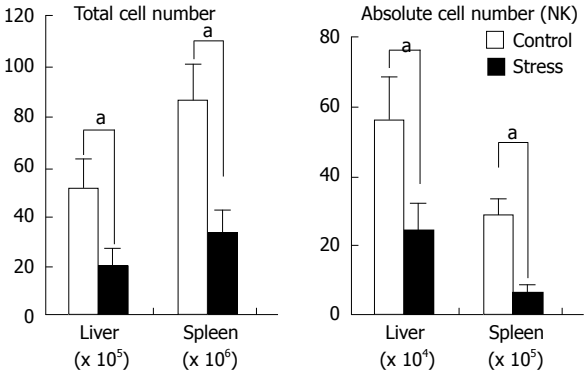

Figure 2 The number of lymphocytes and of natural killer cells in mouse liver and spleen after 24 h of restraint stress. NK: Natural killer. $^{a}P < 0.05$ *vs* control group.

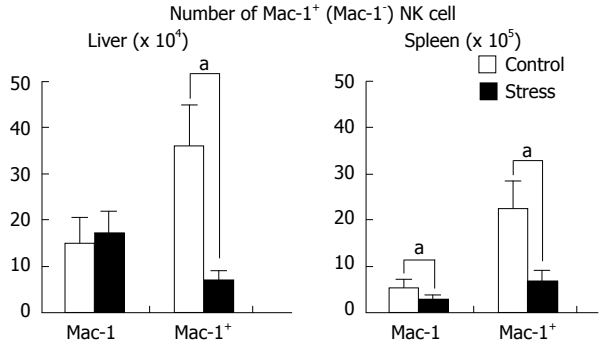

Figure 3 Determination of macrophage-1^{+} and macrophage-1^{-} natural killer cells in mouse liver and spleen after 24 h of restraint stress. Mac-1^{+}: Macrophage-1 positive; Mac-1^{-}: Macrophage-1 negative; NK: Natural killer. $^{a}P < 0.05$ *vs* control group.

autoregulation and cause psychosomatic damage[13,14].

Numerous studies have demonstrated that acute stress stimulation causes distinctly reduced number of lymphocytes in the thymus, spleen, peripheral blood, and liver, as well as disrupt the function of T, B and NK cells. Moreover, the decrease in lymphocytes mainly results from apoptosis.

Our research shows that the number of splenic lymphocytes was significantly decreased, but their percentage did not change significantly. We speculate that various lymphocytes in spleen decreased with the same percentage, which is consistent with the results of previous studies[10,15]. Moreover, the results showed that the number of hepatic lymphocytes significantly declined but the percentage of NK cells dramatically increased (Figures 1A and 2). Therefore, the number of NK cells in the liver and the spleen were determined. Although both the liver and the spleen had fewer NK cells, the number of NK cells was relatively high in the liver, dramatically increasing the percentage of NK cells in the liver (Figure 2). These results indicate that the hepatic NK cells differed from splenic NK cells after stress stimulation. A recent study reported that NK cells have organ specificity, which allows liver to be considered as immune organ and NK cells possess unique functional characteristics[16,17].

Mac-1 was employed to distinguish the subtypes of NK cells with different functions. Mac-1 (CD11b/CD18) is an adhesion molecule of the integrin family, highly expressed in most myeloid hematopoietic cells, such as neutrophils, monocytes/macrophages, eosinophils and B cells. Mac-1 is closely correlated with cell phagocytosis and adhesion, as well as a marker for myeloid and lymphoid hematopoietic cells. NK cells are the only lymphoid cells that express Mac-1. Some researchers have considered Mac-1 as a marker for mature NK cells, which have cell killing activity and are able to secrete cytokines, whereas Mac-1^{-} NK cells are immature, with limited cell killing activity and cytokine production[18-20]. Mac-1 is expressed by 80% to 90% of mature NK cells in the liver, spleen, and peripheral blood. Therefore, current research on NK cells is mainly focused on Mac-1^{+} NK cells. Our previous study discovered numerous Mac-1^{-} NK cells in the liver and demonstrated that Mac-1^{+} NK cells and Mac-1^{-} NK cells have different functional characteristics and cell phenotypes[11]. Therefore, hepatic NK cells were classified into Mac-1^{+} and Mac-1^{-} subtypes according to Mac-1 expression.

Our study shows that the number of Mac-1^{+} and Mac-1^{-} NK cells in the spleen deceases with same percentage after stress stimulation, which results in sustained Mac-1 expression in splenic NK cells. The number of hepatic Mac-1^{+} NK cells significantly decreased, whereas the number of Mac-1^{-} NK cells did not change significantly, which accounts for the reduced Mac-1 expression in hepatic NK cells (Figures 1B and 3). Therefore, we speculate that Mac-1^{-} hepatic NK cells are resistant to the apoptosis induced by stress stimulation.

A large number of studies have demonstrated that acute stress stimulation causes distinctly reduced number of lymphocytes in the thymus, spleen, peripheral blood, and liver, as well as disrupts the function of T, B and NK cells. Moreover, the decrease in lymphocytes is mainly caused by the apoptosis of lymphocytes.

Intracellular Annexin V expression was measured to assess the degree of apoptosis of hepatic NK cells induced by stress stimulation[21]. The results revealed significant NK cell apoptosis, especially Mac-1^{+} NK cells, whereas Mac-1^{-} NK cell apoptosis remained unchanged (Figure 4).

To determine the effects of stress on NK cell function, we employed YAC-1 as target cells to measure killing activity of NK cells. The results imply that stress stimulation decreases the killing activity of splenic NK cells, which accords with the results of previous research. Our study also discovered that stress stimulation does not affect the killing activity of hepatic NK cells, which contrasts with the conclusion of previous investigations. We concluded that Mac-1^{+} NK cells had stronger killing activity than that of Mac-1^{-} NK cells[11]. We also believed that the number of hepatic Mac-1^{+} NK cells declined because of cell apoptosis, which allowed apoptosis-resistant Mac-1^{-} NK cells to survive, and to exhibit relatively strong cell killing activity.

In conclusion, our research preliminarily demon-

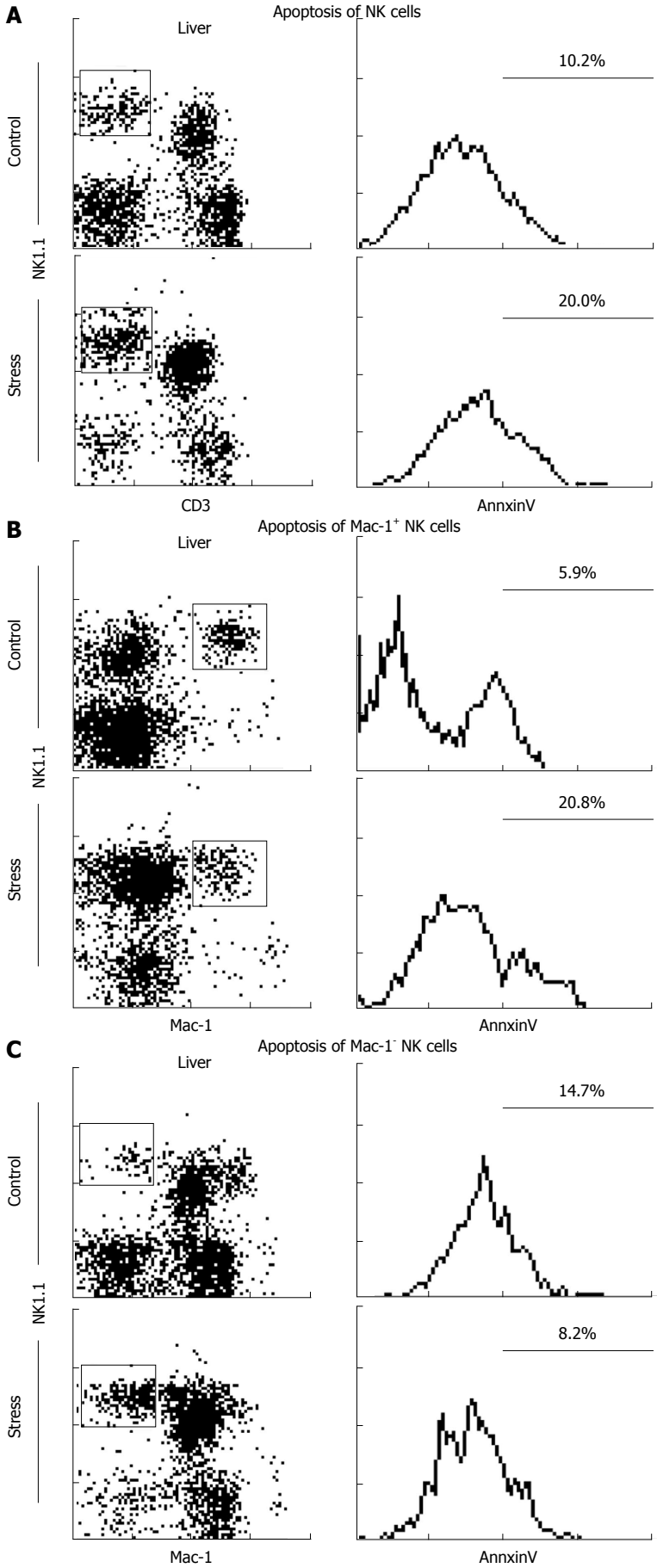

Figure 4 Apoptosis of natural killer cells after 24 h of restraint stress. A: Apoptosis of NK cells; B: Apoptosis of Mac-1$^+$ NK cells; C: Apoptosis of Mac-1$^-$ NK cells. NK: Natural killer; Mac-1: Macrophage-1.

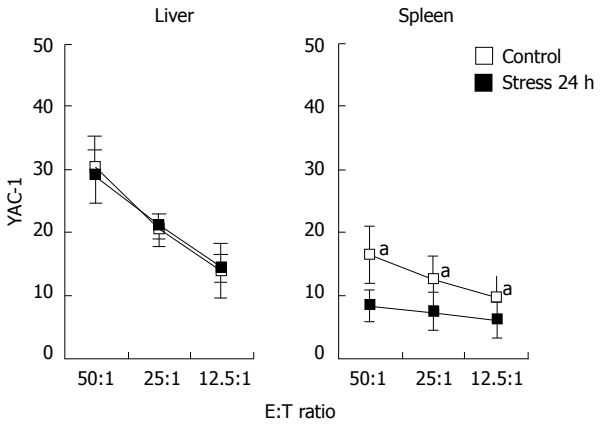

Figure 5 Cytotoxicity of natural killer cells in mouse liver and spleen after 24 h of restraint stress. NK: Natural killer; E:T Ratio: Effector-target ratio. [a]*P* < 0.05 *vs* spleen after 24 h of stress.

strates that intense stress stimulation induces the apoptosis of Mac-1$^+$ hepatic NK cells instead of Mac-1$^-$ NK cells, which requires further investigation to understand the underlying mechanisms and the role of the liver in stress-triggered immune function.

COMMENTS

Background

Stress refers to non-specific systemic reactions to strong stimulus on the body. Intense and prolonged stress stimulation causes immune function disorders, volume shrinkage, and dysfunction of immune organs such as the liver, spleen, and thymus gland. In addition, stress stimulation causes the apoptosis and dysfunction of lymphocytes such as natural killer (NK), T, and B cells in the peripheral blood and immune organs. Most studies have demonstrated that strong stress stimulation may decreases NK cell killing activity in the peripheral blood and spleen, which weakens the immune system.

Research frontiers

NK cells are a class of lymphocytes in the innate immune system that account for about 10% to 15% of all lymphocytes. They have anti-infective, anti-tumour, and immunomodulatory functions, and they regulate hematopoiesis. NK cells mainly function in killing cells and cytokine secretion. NK cells are divided into two subtypes according to surface antigens and functional cell expression, namely, NK_1 and NK_2. Previous investigations have studied NK cells in the peripheral blood and the spleen. The results confirm that the liver generates NK cells during embryogenesis and the function of NK cells in the liver is different from that in the peripheral blood and the spleen.

Innovations and breakthroughs

This study confirms that stress stimulation significantly decreases the number of splenic NK cells, with significantly decreased killing activity, whereas some NK cells survive in the liver. Further research proves that these surviving cells are macrophage-1 (Mac-1)$^-$ NK cells that resist stress-induced cell apoptosis. By contrast, the killing activity of Mac-1$^-$ NK cells is unaffected by stress stimulation.

Applications

This study proves the anti-stress ability of Mac-1$^-$ hepatic NK cells. This finding suggests that Mac-1$^-$ NK cells maintain immune functional stability under stress conditions. Further studies should investigate how to characterize Mac-1$^-$ NK cells and utilize them for preventing the immune dysfunction caused by stress.

Terminology

NK cells are important immune cells with anti-tumour, anti-viral, and immune regulation function, but also participate in the hypersensitivity and occurrence of autoimmune diseases in some cases. Mac-1 (CD11b/CD18) is an adhesion molecule (integrins), and is expressed in most myeloid hematopoietic cells such as neutrophils, monocyte-macrophages, eosinophils, and B cells.

Peer review

Authors investigated the stress-induced apoptosis of NK cells and the changes in their killing activity in mouse livers. NK cell is an important type of lymphocytes. The authors made an interesting research on NK cell. This study proves the anti-stress ability of Mac-1-hepatic NK cells. This finding suggests that Mac-1-NK cells maintain immune functional stability under stress conditions. Further studies should investigate how to characterize Mac-1-NK cells and utilize them for preventing the immune dysfunction caused by stress.

REFERENCES

1 **Gupta MA**. Review of somatic symptoms in post-traumatic stress disorder. *Int Rev Psychiatry* 2013; **25**: 86-99 [PMID: 23383670 DOI: 10.3109/09540261.2012.736367]
2 **Irwin MR**. Human psychoneuroimmunology: 20 years of discovery. *Brain Behav Immun* 2008; **22**: 129-139 [PMID: 17911004]
3 **Yokoyama M**, Itano Y, Katayama H, Morimatsu H, Takeda Y, Takahashi T, Nagano O, Morita K. The effects of continuous epidural anesthesia and analgesia on stress response and immune function in patients undergoing radical esophagectomy. *Anesth Analg* 2005; **101**: 1521-1527 [PMID: 16244024 DOI: 10.1213/01.ANE.0000184287.15086]
4 **Nakata A**. Psychosocial job stress and immunity: a systematic review. *Methods Mol Biol* 2012; **934**: 39-75 [PMID: 22933140 DOI: 10.1007/978-1-62703-071-7_3]
5 **Seth R**, Tai LH, Falls T, de Souza CT, Bell JC, Carrier M, Atkins H, Boushey R, Auer RA. Surgical stress promotes the development of cancer metastases by a coagulation-dependent mechanism involving natural killer cells in a murine model. *Ann Surg* 2013; **258**: 158-168 [PMID: 23108132]
6 **Reiche EM**, Morimoto HK, Nunes SM. Stress and depression-induced immune dysfunction: implications for the development and progression of cancer. *Int Rev Psychiatry* 2005; **17**: 515-527 [PMID: 16401550]
7 **Vivier E**, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. *Nat Rev Immunol* 2012; **12**: 239-252 [PMID: 22437937 DOI: 10.1038/nri3174]
8 **French AR**, Yokoyama WM. Natural killer cells and viral infections. *Curr Opin Immunol* 2003; **15**: 45-51 [PMID: 12495732]
9 **Bierhaus A**, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, Ferstl R, von Eynatten M, Wendt T, Rudofsky G, Joswig M, Morcos M, Schwaninger M, McEwen B, Kirschbaum C, Nawroth PP. A mechanism converting psychosocial stress into mononuclear cell activation. *Proc Natl Acad Sci USA* 2003; **100**: 1920-1925 [PMID: 12578963 DOI: 10.1073/pnas.0438019100]
10 **Sagiyama K**, Tsuchida M, Kawamura H, Wang S, Li C, Bai X, Nagura T, Nozoe S, Abo T. Age-related bias in function of natural killer T cells and granulocytes after stress: reciprocal association of steroid hormones and sympathetic nerves. *Clin Exp Immunol* 2004; **135**: 56-63 [PMID: 14678265 DOI: 10.1111/j.1365-2249.2004.02340.x]
11 **Bai X**, Wang S, Tomiyama-Miyaji C, Shen J, Taniguchi T, Izumi N, Li C, Bakir HY, Nagura T, Takahashi S, Kawamura T, Iiai T, Okamoto H, Hatakeyama K, Abo T. Transient appearance of hepatic natural killer cells with high cytotoxicity and unique phenotype in very young mice. *Scand J Immunol* 2006; **63**: 275-281 [PMID: 16623927]
12 **Minagawa M**, Oya H, Yamamoto S, Shimizu T, Bannai M, Kawamura H, Hatakeyama K, Abo T. Intensive expansion of natural killer T cells in the early phase of hepatocyte regeneration after partial hepatectomy in mice and its association with sympathetic nerve activation. *Hepatology* 2000; **31**: 907-915 [PMID: 10733547 DOI: 10.1053/he.2000.5850]

13 **Harlan JM**, Vedder NB, Winn RK, Rice CL. Mechanisms and consequences of leukocyte-endothelial interaction. *West J Med* 1991; **155**: 365-369 [PMID: 1685284]
14 **Ross GD**, Vetvicka V, Yan J, Xia Y, Vetvicková J. Therapeutic intervention with complement and beta-glucan in cancer. *Immunopharmacology* 1999; **42**: 61-74 [PMID: 10408367]
15 **Shimizu T**, Kawamura T, Miyaji C, Oya H, Bannai M, Yamamoto S, Weerasinghe A, Halder RC, Watanabe H, Hatakeyama K, Abo T. Resistance of extrathymic T cells to stress and the role of endogenous glucocorticoids in stress associated immunosuppression. *Scand J Immunol* 2000; **51**: 285-292 [PMID: 10736098 DOI: 10.1046/j.1365-3083.2000.00695.x]
16 **Michel T**, Poli A, Domingues O, Mauffray M, Thérésine M, Brons NH, Hentges F, Zimmer J. Mouse lung and spleen natural killer cells have phenotypic and functional differences, in part influenced by macrophages. *PLoS One* 2012; **7**: e51230 [PMID: 23227255 DOI: 10.1371/annotation/93791c32-5d2e-4fbc-bd74-ba219c1bd79a]
17 **Racanelli V**, Rehermann B. The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271 DOI: 10.1002/hep.21060]
18 **Větvicka V**, Hanikýrová M, Větvicková J, Ross GD. Regulation of CR3 (CD11b/CD18)-dependent natural killer (NK) cell cytotoxicity by tumour target cell MHC class I molecules. *Clin Exp Immunol* 1999; **115**: 229-235 [PMID: 9933447]
19 **Werfel T**, Witter W, Götze O. CD11b and CD11c antigens are rapidly increased on human natural killer cells upon activation. *J Immunol* 1991; **147**: 2423-2427 [PMID: 1680915]
20 **Kim S**, Iizuka K, Kang HS, Dokun A, French AR, Greco S, Yokoyama WM. In vivo developmental stages in murine natural killer cell maturation. *Nat Immunol* 2002; **3**: 523-528 [PMID: 12006976 DOI: 10.1038/ni796]
21 **Katsuta M**, Takigawa Y, Kimishima M, Inaoka M, Takahashi R, Shiohara T. NK cells and gamma delta+ T cells are phenotypically and functionally defective due to preferential apoptosis in patients with atopic dermatitis. *J Immunol* 2006; **176**: 7736-7744 [PMID: 16751421]

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BRIEF ARTICLE

Interaction between cyclooxygenase-2, Snail, and E-cadherin in gastric cancer cells

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Abstract

AIM: To investigate the mechanisms of how cyclooxygenase-2 (COX-2) regulates E-cadherin in gastric cancer cells.

METHODS: COX-2 expression in human gastric cancer cell lines SGC-7901, BGC-823, MGC-803 and AGS were measured at the mRNA and protein level. COX-2 rich cell line SGC-7901 was chosen for subsequent experiments. siRNA mediated gene knockdown was used to investigate the impact of COX-2 on nuclear factor-κB (NF-κB), Snail, and E-cadherin in gastric cancer cells. Gene expression was determined by Western blot and real-time polymerase chain reaction. To analyze whether NF-κB inhibition could interrupt the modulatory effect of COX-2 or prostaglandin E2 (PGE2) on E-cadherin, gastric cancer cells were treated with celecoxib or PGE2, in the presence of NF-κB specific siRNA.

RESULTS: Highest expression level of COX-2 was found in SGC-7901 cells, both at mRNA and protein levels. siRNA mediated down-regulation of COX-2 led to a reduced expression of NF-κB and Snail, but an increased expression of E-cadherin in SGC-7901 cells. siRNA mediated down-regulation of NF-κB also led to a reduced expression of E-cadherin and Snail in SGC-7901 cells. However, COX-2 expression did not alter after cells were treated with NF-κB specific siRNA in SGC-7901 cells. Treatment of SGC-7901 cells with celecoxib led to a reduced expression of Snail but an increased expression of E-cadherin. In contrast, treatment of SGC-7901 cells with PGE2 led to an increased Snail and a decreased E-cadherin. However, siRNA-mediated knockdown of NF-κB partially abolished the effect of celecoxib and PGE2 on the regulation of E-cadherin and Snail in SGC-7901 cells.

CONCLUSION: COX-2 likely functions upstream of NF-κB and regulates the expression of E-cadherin *via* NF-κB/Snail signaling pathway in gastric cancer cells.

Key words: Cyclooxygenase-2; E-cadherin; celecoxib; Prostaglandin E2; Gastric cancer

Core tip: Cyclooxygenase-2 (COX-2) plays an important role in transcriptional regulation of E-cadherin in gastric cancer and other malignancies. On the contrary, prostaglandin E2 (PGE2) promotes invasion of tumor cells through down-regulating the expression of E-cadherin.

Our study has provided further evidence that COX-2 functions upstream of nuclear factor-κB in the regulation of Snail and E-cadherin in gastric cancer cells. Blockade of COX-2 activity or inhibition of PGE2 production may offer some benefit in the chemoprevention and treatment of gastric cancer.

Liu XJ, Chen ZF, Li HL, Hu ZN, Liu M, Tian AP, Zhao D, Wu J, Zhou YN, Qiao L. Interaction between cyclooxygenase-2, Snail, and E-cadherin in gastric cancer cells. *World J Gastroenterol* 2013; 19(37): 6265-6271 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6265.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6265

INTRODUCTION

Cyclooxygenase-2 (COX-2) is an inducible isozyme of cyclooxygenase and catalyzes prostaglandin E2 (PGE2) formation in response to various inflammatory stimuli or growth factors[1]. PGE2 plays an important role in regulating diverse cellular functions under physiological and pathological conditions[2]. Overexpression of COX-2 is related to invasion and metastasis of tumor cells[3-5]. To further support the role of COX-2 in tumor promotion, it was reported that PGE2 was able to facilitate the invasion of tumor cells through down-regulation of E-cadherin[6]. On the other hand, celecoxib, a selective inhibitor of COX-2, could inhibit migration and metastasis of tumor cells by up-regulating E-cadherin[7]. Many studies have suggested that COX-2 is generally overexpressed in gastric cancer tissues, and it was thought to play a crucial role in the development and invasion of gastric cancers[8]. In contrast, the expression of E-cadherin, an important cell adhesion molecule, is usually low, mutated, or even lost in gastric cancer tissues[9,10]. Thus, COX-2 and E-cadherin appear to exhibit totally different expression patterns. Our group had previously reported an inverse correlation between COX-2 and E-cadherin and suggested that Snail is likely to be responsible for the regulation of COX-2 on the expression and function of E-cadherin in gastric cancer tissues[11,12].

Snail is a transcription factor and was reported to down-regulate the expression of E-cadherin, causing disruption to cell-to-cell adhesion and thereby facilitates tumor progression and metastases[13,14]. Meanwhile, it was reported that nuclear factor-κB (NF-κB) promotes tumor cell migration and invasion in many human cancers through up-regulating Snail and subsequent suppression of E-cadherin[15-17].

Therefore, it is very likely that the interaction between COX-2, Snail, and E-cadherin may play a key regulatory role in invasion and metastasis of gastric cancer. Our group is interested in understanding the possible interaction between COX-2, Snail, and E-cadherin during the development, progression, invasion, and metastasis of gastric cancer. Thus, the aim of this study is to investigate if COX-2 modulates E-cadherin expression *via* Snail and NF-κB in gastric cancer cells.

MATERIALS AND METHODS

Reagents and cell lines

RPMI 1640 medium and PGE2 were purchased from Sigma-Aldrich (St. Louis; MO, United States). Opti-MEM I Reduced Serum Medium, Lipofectamine 2000, BLOCK-iT™ Fluorescent Oligo, and negative control for RNAi were purchased from Invitrogen (Carlsbad, CA, United States). Fetal calf serum was purchased from Hyclone Laboratories (Logan, UT, United States). Reverse transcription kit and quantitative polymerase chain reaction (qPCR) kit were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). celecoxib was purchased from Cayman Chemical (Ann Arbor, MI, United States). Polyclonal antibodies against COX-2, NF-κB P65, E-cadherin, and β-actin were from BioWorld Corporation (CA, United States). Polyclonal antibody against human Snail was purchased from Abcam (Cambridge, United Kingdom). All primers were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). Double strand (ds) RNAi Stealth™ oligos, the specific siRNA against COX-2 and NF-κB (p65) were designed and synthesized by Invitrogen (Carlsbad, CA, United States).

Human gastric cancer cell lines SGC-7901, BGC-823, MGC-803 and AGS were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

Cell culture

Gastric cancer cell lines (SGC-7901, BGC-823, MGC-803 and AGS) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, and maintained at 37 ℃ in a humidified atmosphere containing 50 mL/L CO_2. Before transfection, the culture medium RPMI 1640 was replaced by Opti-MEM I.

Baseline expression of COX-2 in gastric cancer cells

SGC-7901, BGC-823, MGC-803 and AGS were plated respectively at a concentration of 10^5 cells/ well in a 6-well plate and incubated overnight. Total RNA and protein were exacted to determine the basal expression level of COX-2 at the mRNA by PCR and protein level by Western blot, respectively.

siRNAs design, transient transfection of SGC-7901 cells with COX-2 and NF-κB siRNA oligonucleotides

As the SGC-7901 cells showed the highest expression level of COX-2, we used siRNA knockdown approach to investigate the impact of COX-2 on NF-κB, Snail, and E-cadherin in this cell line. Three pairs of siRNA oligos against COX-2 and NF-κB p65, and a control (scrambled) siRNA were initially designed and commercially synthesized. The sequences of these siRNAs were shown in Table 1.

For transfection, cells were seeded into a 6-well plate at a density of 3×10^5 cells per well and incubated overnight. Cells were then transfected with siRNA oligos

Table 1 Sequences of the specific siRNA against cyclooxygenase-2 and nuclear factor-κB p65 used in the study

siRNA against	Forward	Reverse
COX-2	AAUAGGAGAG-GUUAGAGAAGGCUUC	GAAGSCUUCUCUAAC-CUCUCCUAUU
NF-κB p65	UCACUAGGC-GAGUUAUAGSCUCAGG	CCUGAGGCUAUAA-CUCGCCUAGUGA

COX-2: Cyclooxygenase-2; NF-κB: Nuclear factor-κB.

using Lipofectamine 2000 and incubated for 24 to 72 h before further analysis. Transfection efficiency was determined by transfecting the cells with FITC labeled Oligo and counting the number of positive cells under the fluorescent microscopy. More than 80% of cells were routinely successfully transfected. The expression of COX-2, NF-κB, Snail and E-cadherin were analyzed by qPCR and Western blot in successfully transfected cells.

Co-treatment of SGC-7901 cells with NF-κB specific siRNA, celecoxib, and PGE2

To analyze whether NF-κB inhibition could interrupt the modulation effect of COX-2 or PGE2 on E-Cadheirn, SGC-7901 cells were treated with 40 μmol/L celecoxib for 24 h alone or with NF-κB specific siRNA. Cells were also treated with 10 μmol/L PGE2 for 4 h alone or with NF-κB specific siRNA. The optimal dosages for celecoxib and PGE2 were based on our preliminary study. The expression of NF-κB, Snail and E-cadherin were measured by qPCR and Western blot.

Western blotting for COX-2, NF-κB, Snail and E-cadherin expression

Whole-cell extracts were prepared from the treated cells with 2 mL of RIPA buffer containing protease inhibitors. Cell lysates were centrifuged at 8000 rpm for 10 min and the supernatant was collected. Cell lysates were electrophoretically separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels. Proteins were transferred to nitrocellulose membrane and the membrane was blocked with 5% fat-free milk in TBS plus 0.1% Tween-20 (TBST). The membranes were then incubated with respective primary antibodies (rabbit polyclonal COX-2, NF-κB p65, Snail, E-cadherin, all at 1:1000 dilution) and the corresponding horseradish peroxidase-conjugated secondary antibody for 1 h. The membranes were incubated with enhanced chemiluminescence system and exposed to X-ray film for signal detection. β-actin was used as a control for equal loading of samples.

Real-time PCR for COX-2, NF-κB, Snail and E-cadherin expression

Total RNA was extracted with Trizol reagent according to the manufacturer's instructions. Approximately 30 ng of total RNA was transcribed into cDNA. The synthesized cDNA samples were subjected to qPCR using SYBR® Green Quantitative PCR kit. Amplification was carried out in a total volume of 20 μL for 40 cycles of 15 s at 95 ℃, 20 s at 60 ℃, and 30 s at 72 ℃. Samples were run in triplicate and their relative expression was determined by normalizing expression of each target to β-actin. The amplification was monitored on a Roter-Gene realtime PCR apparatus (Roter-Gene, Australia). Primers used in these experiments were shown in Table 2.

Table 2 The sequences of the primers used in this study

Primers	Sense primer	Antisense primer
COX-2	5'-GCCTGAATGTGCCATA AGACTGAC-3'	5'-AAACCCACAGTGCTTG ACACAGA-3'
E-cadherin	5'-TACACTGCCCAGGAGS CAGA-3′	5'-TGGCACCAGTGTCCGG ATTA-3′
Snail	5'-GACCACTATGCCGCGC TCTT-3′	5'-TCGCTGTAGTTAGGCT TCCGATT-3′
NF-κB p65	5'-TCAGTCAGSGCATCCA GACC-3′	5'-CAGAGSCGCACAGSAT TCA-3′
β-actin	5'-TGGCACCCAGSACAAT GAA-3'	5'-CTAAGTCATAGTCCGC CTAGAAGSA-3'

COX-2: Cyclooxygenase-2; NF-κB: Nuclear factor-κB.

Statistical analysis

Data analysis was performed using SPSS11.0. All data were expressed as mean ± SD. Comparison of the differences between each group was performed by χ^2 test. A P value of < 0.05 was considered statistically significant.

RESULTS

COX-2 baseline expression in human gastric cancer cell lines

We first examined the basal level of COX-2 expression in several human gastric cancer cell lines using qPCR and Western blot. The cell lines tested include SGC-7901 (moderately differentiated), BGC-823 (poorly differentiated), MGC803 (undifferentiated), and AGS (well differentiated). Highest expression level of COX-2 was found in SGC-7901 cells, both at mRNA and protein levels (Figure 1A and B) ($P < 0.05$). Thus, the subsequent experiments were performed in SGC-7901 unless otherwise stated.

Effect of COX-2 silencing on NF-κB, Snail, and E-cadherin in SGC7901 cells

In order to test the effect of siRNA-mediated down-regulation of COX-2 on NF-κB, Snail and E-cadherin, SGC-7091 cells were incubated with COX-2 specific siRNA (COX-2-siRNA) and the target gene expression was examined by qPCR and Western blot. As shown in Figure 1, knockdown of COX-2 (Figure 1C and D) led to a 3-fold and 2.3-fold decrease but a 4.6-fold increase in the mRNA expression of NF-κB, Snail, and E-cadherin, respectively (Figure 1E) ($P < 0.05$, compared to their respective controls). These changes were confirmed at the protein level: COX-2-siRNA led to 2.3-fold and 2.8-fold

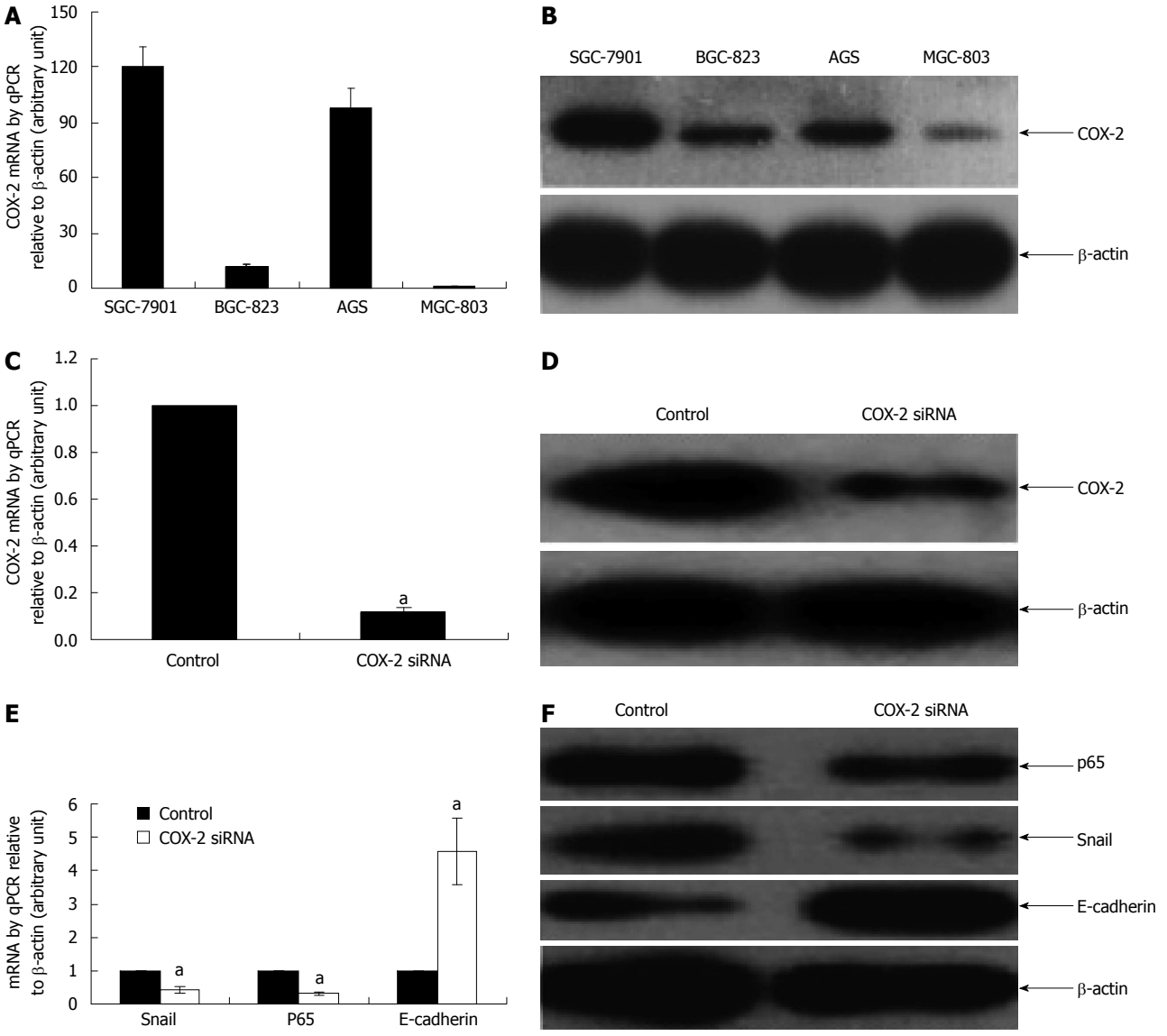

Figure 1 Effect of cyclooxygenase-2 knockdown on the expression of nuclear factor-κB, Snail, and E-cadherin in gastric cancer cells. Among the four human gastric cancer cell lines, SGC-7901 has the highest expression level of cyclooxygenase-2 (COX-2) at mRNA (A) and protein level (B). Thus, this cell line was used to study the regulatory effect of COX-2 on nuclear factor-κB (NF-κB), Snail, and E-cadherin. Successful knockdown of COX-2 was confirmed at mRNA (C) and protein (D) levels. Down-regulation of COX-2 led to a reduction of NF-κB subunit p65 and Snail but an increased E-Cadherin, both at the mRNA (E) and protein (F) levels. mRNA expression was examined by quantitative polymerase chain reaction (qPCR) and expressed as a relative arbitrary unit against that of β-actin. Protein expression was examined by Western blot ($^aP < 0.05$ *vs* their respective controls).

decrease but a 2.5-fold increase in the expression of NF-κB, Snail, and E-cadherin, respectively (Figure 1F) ($P < 0.05$, compared to their respective controls).

Effect of NF-κB silencing on COX-2, Snail and E-cadherin in SGC7901 cells

As noted above, siRNA mediated down-regulation of COX-2 led to a reduced expression of NF-κB and Snail in SGC-7901 cells. In order to confirm if COX-2 functions upstream of NF-κB, we examined if NF-κB was able to modulate COX-2 expression in SGC-7901 cells. As shown in Figure 2, knockdown of NF-κB subunit p65 using its specific siRNA (p65-siRNA) (Figure 2A and B) did not affect the expression of COX-2 at both mRNA and protein levels (Figure 2C and D) ($P < 0.05$, compared to their respective controls).

We then proposed that NF-κB could regulate the expression of E-cadherin *via* the transcription factor Snail. Therefore, the effect of NF-κB silencing on Snail and E-cadherin were further examined in SGC-7091 cells. As shown in Figure 2, knockdown of NF-κB (Figure 2A and B) was associated with a reduced expression of Snail at both mRNA and protein levels (Figure 2C and D) ($P < 0.05$, compared to their respective controls). On the other hand, blockade of NF-κB with p65-siRNA rendered an increase in the expression of E-cadherin at both mRNA and protein levels (Figure 2C and D) ($P < 0.05$, compared to their respective controls).

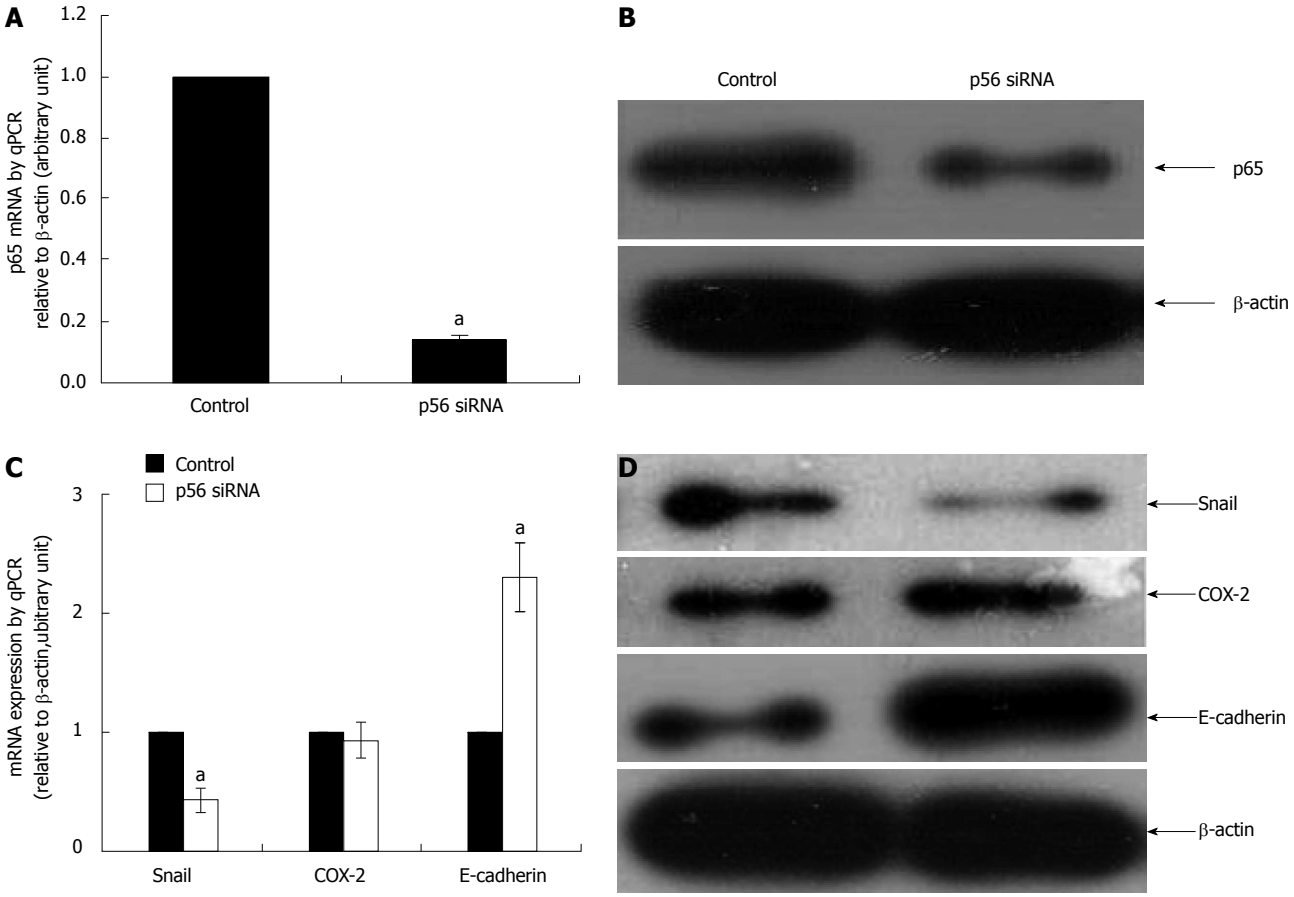

Figure 2 Effect of nuclear factor-κB knockdown on the expression of cyclooxygenase-2, Snail, and E-cadherin in SGC-7901 cells. Cells transfected with specific siRNA against nuclear factor-κB (NF-κB) subunit p65 (p65-siRNA) showed a marked down-regulation of p65 at mRNA (A) and protein (B) levels. p65-siRNA led to a reduction of Snail but an increased E-cadherin, both at the mRNA (C) and protein (D) levels. However, p65-siRNA mediated down-regulation of NF-κB did not significantly alter the expression of COX-2, both at the mRNA (C) and protein (D) levels. mRNA expression was examined by polymerase chain reaction (qPCR) and expressed as a relative arbitrary unit against that of β-actin. Protein expression was examined by Western blot. ($^aP < 0.05$ *vs* their respective controls).

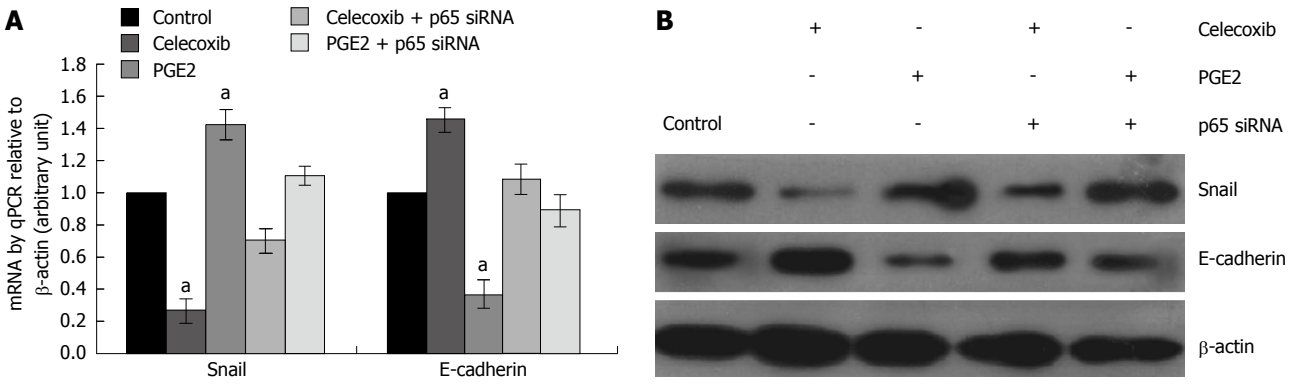

Figure 3 Down-regulation of nuclear factor-κB by p65-siRNA reversed the regulatory effect of celecoxib and prostaglandin E2 on Snail and E-cadherin in SGC-7901 cells. Treatment of SGC-7901 cells with celecoxib led to a reduced expression of Snail but an increased expression of E-cadherin both at mRNA (A) and protein (B) levels. In contrast, treatment of SGC-7901 cells with prostaglandin E2 (PGE2) led to an increased Snail and a decreased E-cadherin at mRNA (A) and protein (B) levels. However, when the cells were pre-treated with p65-siRNA, the observed effects of Celecoxib and PGE2 were reversed (A, B). mRNA expression was examined by polymerase chain reaction (qPCR) and expressed as a relative arbitrary unit against that of β-actin. Protein expression was examined by Western blot ($^aP < 0.05$ *vs* their respective controls).

NF-κB inhibition interrupted the effects of celecoxib and PGE2 on E-cadherin and Snail in SGC-7901 cells

To further determine the regulatory role of NF-κB on E-cadherin, we used celecoxib, a potent COX-2 inhibitor, and PGE2, a principal COX-2 substrate with reported role in promoting cell migration and invasion in tumors, to treat SGC-7901 cells in the presence or absence of p65-siRNA.

As shown in Figure 3, treatment of SGC-7901 cells with celecoxib led to a reduced expression of Snail but an increased expression of E-cadherin both at mRNA (Figure 3A) and protein (Figure 3B) levels. In contrast, treatment of SGC-7901 cells with PGE2 led to an increased Snail and a decreased E-cadherin at mRNA (Figure 3A) and protein levels (Figure 3B). However, when the cells were pre-treated with p65-siRNA, the observed effects of celecoxib and PGE2 were reversed (Figure 3A and B) ($P < 0.05$, compared to their respective controls).

DISCUSSION

Abnormal down-regulation of E-cadherin is an important event involved in epithelial-mesenchymal transition, a critical process in the malignant transformation of epithelial cancers including gastric cancer[10,18]. Previous studies, including our own, have demonstrated that COX-2 has a modulatory effect on expression of E-cadherin in gastric cancer and other malignancies[12]. The regulatory role of COX-2 on the expression of E-cadherin is also reflected by the observed chemopreventive effect of the selective COX-2 inhibitor celecoxib which was shown to inhibit the migration and metastasis of tumor cells by up-regulating E-cadherin[19], and further supported by the fact that PGE-2 was able to promote the tumor invasion through down-regulating E-cadherin[20]. However, the mechanisms responsible for the regulatory effect of COX-2 on E-cadherin have not been well defined.

E-cadherin is usually lost in gastric cancer tissues and this appeared to be mediated by COX-2[21]. In our previous study, we found that inhibition of COX-2 activity by celecoxib was not only associated with a reduced expression of Snail, but also a marked reduction in NF-κB subunit p65[12]. In the current study, we explored the same regulatory effect based on RNAi technique. The results showed that COX-2 mediated down-regulation of E-cadherin appeared to be dependent on a functional NF-κB pathway, as blockade of COX-2 activity, either by COX-2-siRNA or celecoxib, restored the expression of E-cadherin. This was associated with a marked down-regulation of NF-κB and Snail expression. These findings are in agreement with previous reports that NF-κB up-regulates Snail and consequently represses E-cadherin in tumor cells[21,22]. Snail has been firmly established as a repressor of E-cadherin and it down-regulates E-cadherin transcription through an interaction with proximal E-boxes of the E-cadherin promoter[23]. In our current study, we further revealed that blockade of NF-κB by p65-siRNA did not alter the expression of COX-2 in SGC-7901 cells. However, the effect of celecoxib and PGE2 on Snail and E-cadherin was reversed by p65-siRNA, suggesting that a functional COX-2 was necessary for regulating NF-κB and Snail signaling in gastric cancer.

The regulatory role of NF-κB on COX-2 has been reported in other human tumors[24]. For example, NF-κB was found to enhance the expression of COX-2 and promote cells proliferation in human colorectal carcinoma cells[25]. In our study, NF-κB p65 was not found to regulate the expression of COX-2. This inconsistency may reflect a cell type specific difference. Additionally, the regulatory role of NF-κB on COX-2 in gastric cancer through other subunits could not be excluded. More studies are needed to unveil the possible mechanisms of how COX-2 and NF-κB interact during gastric cancer formation.

In conclusion, this study has provided further evidence that COX-2 functions upstream of NF-κB in the regulation of Snail and E-cadherin in gastric cancer cells. Blockade of COX-2 activity or inhibition of PGE2 production may offer some benefit in the chemoprevention and treatment of gastric cancer.

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COMMENTS

Background

Cyclooxygenase-2 (COX-2) plays an important role in transcriptional regulation of E-cadherin in gastric cancer and other malignancies. celecoxib, a selective inhibitor of COX-2, inhibits migration and metastasis of tumor cells by up-regulation of E-cadherin. On the contrary, prostaglandin E2 (PGE2) promotes invasion of tumor cells through down regulating the expression of E-cadherin. This study aims to explore how COX-2/PGE2 regulates E-cadherin expression and further to determine whether COX-2/PGE2 reduces the expression of E-cadherin *via* nuclear factor-κB (NF-κB)/ Snail signal pathway in gastric cancer cells.

Research frontiers

Although the correlation between COX-2 and E-cadherin is always inverse in tumor cells, the mechanism of how COX-2 regulates E-cadherin is not clear yet.

Innovations and breakthroughs

The authors firstly found COX-2 baseline expression was significantly higher in SGC-7901 cells in comparison to that in BGC-823, MGC-803 and AGS cells. celecoxib or COX-2 specific RNAi both down-regulated NF-κB and Snail expression, and up-regulated E-cadherin expression, in contrast to PGE2, in SGC-7901 cells. Next, they found that NF-κB specific RNAi did not influence the expression of COX-2 in SGC-7901 cells. Therefore, they can conclude preliminarily that NF-κB and Snail are the downstream molecules in COX-2 modulated E-cadherin signaling pathway in SGC-7901 cells.

Applications

This study has provided further evidence that COX-2 functions upstream of NF-κB in the regulation of Snail and E-cadherin in gastric cancer cells. Blockade of COX-2 activity or inhibition of PGE2 production may offer some benefit in the chemoprevention and treatment of gastric cancer.

Terminology

Epithelial-mesenchymal transition (EMT): The epithelial-mesenchymal transition is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. EMT is essential for numerous developmental processes including mesoderm formation and neural tube formation. EMT has also been shown to occur in wound healing, in organ fibrosis and in the initiation of metastasis for cancer progression. COX-2: COX-2 is an inducible isozyme of cyclooxygenase and catalyzes PGE2 formation in response to various inflammatory stimuli or growth factors; E-cadherin: E-cadherin is an important cell adhesion molecule and is usually low, mutated, or even lost in gastric cancer tissues. COX-2 and E-cadherin appear to exhibit totally different expression patterns in tumor cells.

Peer review

This is a very interesting paper on the molecular biology of COX-2 and E-cadherin *via* the NF-κB and Snail pathways. The methodology and reasoning is

sound along with the results and logical discussion at the end.

REFERENCES

1 **Nakao S**, Ogtata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H. Tumor necrosis factor alpha (TNF-alpha)-induced prostaglandin E2 release is mediated by the activation of cyclooxygenase-2 (COX-2) transcription via NFkappaB in human gingival fibroblasts. *Mol Cell Biochem* 2002; **238**: 11-18 [PMID: 12349897]

2 **Takehara H**, Iwamoto J, Mizokami Y, Takahashi K, Ootubo T, Miura S, Narasaka T, Takeyama H, Omata T, Shimokobe K, Ito M, Matsuoka T. Involvement of cyclooxygenase-2--prostaglandin E2 pathway in interleukin-8 production in gastric cancer cells. *Dig Dis Sci* 2006; **51**: 2188-2197 [PMID: 17078003 DOI: 10.1007/s10620-006-9436-2]

3 **Costa C**, Soares R, Reis-Filho JS, Leitão D, Amendoeira I, Schmitt FC. Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. *J Clin Pathol* 2002; **55**: 429-434 [PMID: 12037025]

4 **Leung WK**, To KF, Go MY, Chan KK, Chan FK, Ng EK, Chung SC, Sung JJ. Cyclooxygenase-2 upregulates vascular endothelial growth factor expression and angiogenesis in human gastric carcinoma. *Int J Oncol* 2003; **23**: 1317-1322 [PMID: 14532971]

5 **Huang DS**, Shen KZ, Wei JF, Liang TB, Zheng SS, Xie HY. Specific COX-2 inhibitor NS398 induces apoptosis in human liver cancer cell line HepG2 through BCL-2. *World J Gastroenterol* 2005; **11**: 204-207 [PMID: 15633216]

6 **Hu Z**, Liu X, Tang Z, Zhou Y, Qiao L. Possible regulatory role of Snail in NF-κB-mediated changes in E-cadherin in gastric cancer. *Oncol Rep* 2013; **29**: 993-1000 [PMID: 23254865 DOI: 10.3892/or.2012.2200]

7 **Zhou Y**, Ran J, Tang C, Wu J, Honghua L, Xingwen L, Ning C, Qiao L. Effect of celecoxib on E-cadherin, VEGF, Microvessel density and apoptosis in gastric cancer. *Cancer Biol Ther* 2007; **6**: 269-275 [PMID: 17224647]

8 **Joo YE**, Rew JS, Seo YH, Choi SK, Kim YJ, Park CS, Kim SJ. Cyclooxygenase-2 overexpression correlates with vascular endothelial growth factor expression and tumor angiogenesis in gastric cancer. *J Clin Gastroenterol* 2003; **37**: 28-33 [PMID: 12811205]

9 **Bremnes RM**, Veve R, Hirsch FR, Franklin WA. The E-cadherin cell-cell adhesion complex and lung cancer invasion, metastasis, and prognosis. *Lung Cancer* 2002; **36**: 115-124 [PMID: 11955645]

10 **Onder TT**, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res* 2008; **68**: 3645-3654 [PMID: 18483246 DOI: 10.1158/0008-5472.CAN-07-2938]

11 **Zhou Y**, Li G, Wu J, Zhang Z, Wu Z, Fan P, Hao T, Zhang X, Li M, Zhang F, Li Q, Lu B, Qiao L. Clinicopathological significance of E-cadherin, VEGF, and MMPs in gastric cancer. *Tumour Biol* 2010; **31**: 549-558 [PMID: 20563765 DOI: 10.1007/s13277-010-0068-y]

12 **Chen Z**, Liu M, Liu X, Huang S, Li L, Song B, Li H, Ren Q, Hu Z, Zhou Y, Qiao L. COX-2 regulates E-cadherin expression through the NF-κB/Snail signaling pathway in gastric cancer. *Int J Mol Med* 2013; **32**: 93-100 [PMID: 23670240 DOI: 10.3892/ijmm.2013.1376]

13 **Bolós V**, Peinado H, Pérez-Moreno MA, Fraga MF, Esteller M, Cano A. The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. *J Cell Sci* 2003; **116**: 499-511 [PMID: 12508111 DOI: 10.1242/jcs.00224]

14 **Peinado H**, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. *Mol Cell Biol* 2004; **24**: 306-319 [PMID: 14673164]

15 **Baranwal S**, Alahari SK. Molecular mechanisms controlling E-cadherin expression in breast cancer. *Biochem Biophys Res Commun* 2009; **384**: 6-11 [PMID: 19379710 DOI: 10.1016/j.bbrc.2009.04.051]

16 **Kojima M**, Morisaki T, Sasaki N, Nakano K, Mibu R, Tanaka M, Katano M. Increased nuclear factor-kB activation in human colorectal carcinoma and its correlation with tumor progression. *Anticancer Res* 2004; **24**: 675-681 [PMID: 15161011]

17 **De Craene B**, Gilbert B, Stove C, Bruyneel E, van Roy F, Berx G. The transcription factor snail induces tumor cell invasion through modulation of the epithelial cell differentiation program. *Cancer Res* 2005; **65**: 6237-6244 [PMID: 16024625 DOI: 10.1158/0008-5472.CAN-04-3545]

18 **Natalwala A**, Spychal R, Tselepis C. Epithelial-mesenchymal transition mediated tumourigenesis in the gastrointestinal tract. *World J Gastroenterol* 2008; **14**: 3792-3797 [PMID: 18609701]

19 **Dohadwala M**, Yang SC, Luo J, Sharma S, Batra RK, Huang M, Lin Y, Goodglick L, Krysan K, Fishbein MC, Hong L, Lai C, Cameron RB, Gemmill RM, Drabkin HA, Dubinett SM. Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin E(2) induces transcriptional repressors ZEB1 and snail in non-small cell lung cancer. *Cancer Res* 2006; **66**: 5338-5345 [PMID: 16707460 DOI: 10.1158/0008-5472.CAN-05-3635]

20 **Jee YS**, Jang TJ, Jung KH. Prostaglandin E(2) and interleukin-1β reduce E-cadherin expression by enhancing snail expression in gastric cancer cells. *J Korean Med Sci* 2012; **27**: 987-992 [PMID: 22969242 DOI: 10.3346/jkms.2012.27.9.987]

21 **Rao DS**, Gui D, Koski ME, Popoviciu LM, Wang H, Reiter RE, Said JW. An inverse relation between COX-2 and E-cadherin expression correlates with aggressive histologic features in prostate cancer. *Appl Immunohistochem Mol Morphol* 2006; **14**: 375-383 [PMID: 17122632 DOI: 10.1097/01.pai.0000210417.61117.6c]

22 **Julien S**, Puig I, Caretti E, Bonaventure J, Nelles L, van Roy F, Dargemont C, de Herreros AG, Bellacosa A, Larue L. Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. *Oncogene* 2007; **26**: 7445-7456 [PMID: 17563753 DOI: 10.1038/sj.onc.1210546]

23 **Castro Alves C**, Rosivatz E, Schott C, Hollweck R, Becker I, Sarbia M, Carneiro F, Becker KF. Slug is overexpressed in gastric carcinomas and may act synergistically with SIP1 and Snail in the down-regulation of E-cadherin. *J Pathol* 2007; **211**: 507-515 [PMID: 17299729 DOI: 10.1002/path.2138]

24 **Zhang K**, Zhaos J, Liu X, Yan B, Chen D, Gao Y, Hu X, Liu S, Zhang D, Zhou C. Activation of NF-B upregulates Snail and consequent repression of E-cadherin in cholangiocarcinoma cell invasion. *Hepatogastroenterology* 2011; **58**: 1-7 [PMID: 21510277]

25 **Kojima M**, Morisaki T, Izuhara K, Uchiyama A, Matsunari Y, Katano M, Tanaka M. Lipopolysaccharide increases cyclo-oxygenase-2 expression in a colon carcinoma cell line through nuclear factor-kappa B activation. *Oncogene* 2000; **19**: 1225-1231 [PMID: 10713711 DOI: 10.1038/sj.onc.1203427]

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BRIEF ARTICLE

Laparoscopic *vs* open distal pancreatectomy for solid pseudopapillary tumor of the pancreas

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Abstract

AIM: To compare short- and long-term outcomes of laparoscopic *vs* open distal pancreatectomy for solid pseudopapillary tumor (SPT) of the pancreas.

METHODS: This retrospective study included 28 patients who underwent distal pancreatectomy for SPT of the pancreas between 1998 and 2012. The patients were divided into two groups based on the surgical approach: the laparoscopic surgery group and the open surgery group. The patients' demographic data, operative results, pathological reports, hospital courses, morbidity and mortality, and follow-up data were compared between the two groups.

RESULTS: Fifteen patients with SPT of the pancreas underwent laparoscopic distal pancreatectomy (LDP), and 13 underwent open distal pancreatectomy (ODP). Baseline characteristics were similar between the two groups except for a female predominance in the LDP group (100.0% *vs* 69.2%, $P = 0.035$). Mortality, morbidity (33.3% *vs* 38.5%, $P = 1.000$), pancreatic fistula rates (26.7% *vs* 30.8%, $P = 0.728$), and reoperation rates (0.0% *vs* 7.7%, $P = 0.464$) were similar in the two groups. There were no significant differences in the operating time (171 min *vs* 178 min, $P = 0.755$) between the two groups. The intraoperative blood loss (149 mL *vs* 580 mL, $P = 0.002$), transfusion requirement (6.7% *vs* 46.2%, $P = 0.029$), first flatus time (1.9 d *vs* 3.5 d, $P = 0.000$), diet start time (2.3 d *vs* 4.9 d, $P = 0.000$), and postoperative hospital stay (8.1 d *vs* 12.8 d, $P = 0.029$) were significantly less in the LDP group than in the ODP group. All patients had negative surgical margins at final pathology. There were no significant differences in number of lymph nodes harvested (4.6 *vs* 6.4, $P = 0.549$) between the two groups. The median follow-up was 33 (3-100) mo for the LDP group and 45 (17-127) mo for the ODP group. All patients were alive with one recurrence.

CONCLUSION: LDP for SPT has short-term benefits compared with ODP. Long-term outcomes of LDP are similar to those of ODP.

Key words: Solid pseudopapillary tumor; Pancreatic tumor; Laparoscopic surgery; Distal pancreatectomy

Core tip: Solid pseudopapillary tumor (SPT) of the pancreas is a rare neoplasm. Laparoscopic distal pancreatectomy (LDP) and open distal pancreatectomy (ODP) for SPT have not previously been compared. We compared the short-term and long-term outcomes among patients undergoing either LDP or ODP for SPT. Our results showed that LDP for SPT had the advantages of minimally invasive surgery, less intraoperative blood loss, and rapid recovery. The mortality, morbidity, oncological outcome, and long-term outcome of LDP were similar to those of open surgery.

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INTRODUCTION

Solid pseudopapillary tumor (SPT) of the pancreas is a rare neoplasm, accounting for 0.17%-2.7% of all pancreatic tumors, and affecting predominantly young women[1]. Frantz[2] first described the tumor in 1959 as a papillary tumor of the pancreas. The tumor has been named as a papillary epithelial neoplasm, solid and cystic tumor, solid and papillary tumor, papillary cystic tumor, and solid and papillary epithelial neoplasm depending on its histological features including cystic, solid, and pseudopapillary structures[1,3]. In 1996, the World Health Organization renamed this tumor as SPT[4]. SPT is of unclear histopathogenesis, and low-grade malignancy, malignant degeneration and lymph node metastasis rarely occur[1,5]. Surgical resection of this tumor could result in long-term survival[1].

Laparoscopic resection of the pancreas, including enucleation, pancreaticoduodenectomy, and distal and central pancreatectomy, has been recently described; some of the patients could have benefited from these procedures[6-10]. Until April 2013, about 86 cases of laparoscopic/robot-assisted resection for SPT have been reported in the English-language literature. Most of these are case reports and small series. However, there are few reports comparing short-term and long-term outcomes among patients who underwent laparoscopic distal pancreatectomy (LDP) *vs* open distal pancreatectomy (ODP) for SPT of the pancreas.

The goal of the present study was to compare short-term and long-term outcomes in patients undergoing either LDP or ODP for SPT of the pancreas.

MATERIALS AND METHODS

Patient sample and data collection

Between May 1998 and December 2012, 55 patients underwent pancreatectomy for SPT of the pancreas at Sir Run Run Shaw Hospital, Hangzhou, China. We retrieved 29 patients who underwent distal pancreatectomy. One patient with liver metastasis and colon cancer was excluded from the study, and 28 patients were included in this study. The medical records of all patients were retrospectively reviewed, including demographics, clinical presentation, operative results, hospital course, morbidity and mortality, pathological findings, and long-term follow-up data. The Institutional Review Board of Sir Run Run Shaw Hospital of Zhejiang University approved this study protocol.

Surgical procedure

All operations were performed by four experienced surgeons using our institution's standardized technique. Laparoscopic pancreatic surgery was adopted in 2003 at our institution, therefore, all of the patients who underwent surgery from 1998 to 2003 were included in the open surgery group. After 2003, the surgeons could decide whether to perform laparoscopic or open surgery with the informed consent of the patients.

Operative technique used for distal pancreatectomy

The operative procedure for LDP has been described previously[11,12]. Briefly, the patient was placed in supine position with the head slightly elevated. The surgeon and the second assistant who held the laparoscope stood on the right side of the patient and the first assistant stood on the left. One initial 10-mm trocar was placed for laparoscopy below the umbilicus. A 30-degree telescope was inserted to examine the peritoneal cavity to rule out metastatic disease. After general examination, the other four trocars (one 12 mm, three 5 mm) were inserted into the left upper flank, left flank, right upper flank, and right flank quadrants; and the five trocars were arranged in a V shape. Under pneumoperitoneum, the gastrocolic ligament was divided for entrance to the lesser sac using a harmonic scalpel (Harmonic Ace; Ethicon Endo-Surgery, Cincinnati, OH, United States). The mobilization of the pancreas began at the superior border until the proximal splenic artery was visualized. The pancreas was mobilized at the inferior border to visualize the superior mesenteric and splenic veins. After creating a tunnel behind the neck of the pancreas, the pancreas was transected with an endoscopic linear stapler (Endocutter 60 stapler, white or blue cartridge; Ethicon Endo-Surgery, Cincinnati, OH, United States). For spleen-preserving procedures, the distal pancreas was freely dissected from the splenic vessels by ligation of the small branches connected to the pancreas using small titanium vascular clips or a harmonic scalpel. In the case of DP with splenectomy, the splenic artery and splenic vein were divided. The spleen was resected with the pancreas.

ODP was performed in the same manner as LDP through an upper midline incision. However, a variety of techniques, including suturing and/or stapling, were used to control the pancreas stump, according to the preference of the individual surgeon.

Postoperative management

Diet was started after the first flatus had been passed. Patients were discharged if they considered themselves sufficiently recovered; tolerated food without any significant discomfort; and had no major complications. Postoperative pancreatic fistula was defined as any measurable volume of drainage fluid (amylase > 3 times the upper limit of normal serum value) on or after postoperative day 3[13]. Three different grades of postoperative pancreatic fistula (A-C) were defined according to the clinical impact on the patient's hospital course[13]. Postoperative mortality

Table 1 Baseline characteristics of patients undergoing laparoscopic distal pancreatectomy or open distal pancreatectomy for pancreatic solid pseudopapillary tumor *n* (%)

Characteristics	LDP (*n* = 15)	ODP (*n* = 13)	*P* value
Age (yr)	35.4 ± 13.0	35.2 ± 16.6	0.965
Sex			0.035
Male	0 (0.0)	4 (30.8)	
Female	15 (100.0)	9 (69.2)	
BMI (kg/m^2)	20.8 ± 2.3	22.4 ± 6.1	0.392
Symptoms			0.255
No	10 (66.7)	5 (38.5)	
Yes	5 (33.3)	8 (61.5)	
Comorbidity	4 (26.7)	4 (30.8)	1.000
ASA score			1.000
1	9 (60.0)	8 (61.5)	
2	6 (40.0)	5 (38.5)	
Tumor size (cm)	5.1 ± 1.6	7.7 ± 4.1	0.050
Spleen preservation			0.639
No	13 (86.7)	10 (76.9)	
Yes	2 (13.3)	3 (23.1)	
Combined resection			1.000
Gallbladder	0 (0.0)	1 (7.7)	
Gastric stromal tumor	1 (6.7)	0 (0.0)	

Data are expressed as *n* (%) or mean ± SD or unless otherwise specified. BMI: Body mass index; ASA: American Society of Anesthesiologists; LDP: Laparoscopic distal pancreatectomy; ODP: Open distal pancreatectomy.

was defined as death occurring within 30 d after surgery.

Patient follow-up

Patients were followed up as outpatients by telephone. We included data up to the last follow-up in March 2013. Recurrence or distant metastasis was diagnosed pathologically by surgical resection, biopsy, or cytology and/or radiological examination. The fasting blood glucose level (normal ⩽ 110 mg/dL) was used to evaluate pancreatic endocrine function. The clinical evaluation was used to assess the pancreatic exocrine function. Patients with diarrhea, weight loss, and fatty stools were considered to have pancreatic exocrine insufficiency.

Statistical analysis

Continuous clinicopathological data were expressed as median (range) or mean ± SD as appropriate. Categorical variables were reported as number and percentage. Continuous clinicopathological data were analyzed with Student's *t* test (or Mann-Whitney *U* test). Categorical variables were analyzed with the χ^2 test (or Fisher's exact test). All statistical analyses were performed using SPSS version 16.0. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

Table 1 summarizes the baseline characteristics of the LDP and ODP groups. Fifteen patients underwent LDP and 13 ODP. The two groups were balanced in terms of their baseline characteristics: age, body mass index (BMI), symptoms, comorbidity, American Society of Anesthesiologists (ASA) score, tumor size, spleen preservation rate, and combined resection rate, except for a significant female predominance in the LDP group: 100% women (*n* = 15) compared with 69.2% (*n* = 9) in the ODP group (*P* = 0.035).

Table 2 Surgical outcomes of laparoscopic distal pancreatectomy and open distal pancreatectomy for pancreatic solid pseudopapillary tumor *n* (%)

Outcomes	LDP (*n* = 15)	ODP (*n* = 13)	*P* value
Operating time (min)	171 ± 54	178 ± 75	0.755
EBL (mL)	149 ± 127	580 ± 400	0.002
Transfused patients	1 (6.7)	6 (46.2)	0.029
First flatus time (d)	1.9 ± 0.5	3.5 ± 0.9	0.000
Diet start time (d)	2.3 ± 0.7	4.9 ± 2.1	0.000
Postoperative hospital stay (d)	8.1 ± 1.7	12.8 ± 6.8	0.029
Morbidity	5 (33.3)	5 (38.5)	1.000
Pancreatic fistula	4 (26.7)	4 (30.8)	0.972
Grade A	2 (13.3)	2 (15.4)	
Grade B	0 (0.0)	0 (0.0)	
Grade C	2 (13.3)	2 (15.4)	
Intra-abdominal abscess	1 (6.7)	0 (0.0)	1.000
Pleural effusion	0 (0.0)	1 (7.7)	0.464
Reoperation	0 (0.0)	1 (7.7)	0.464
Percutaneous drainage	2 (13.3)	2 (15.4)	1.000
Mortality	0 (0.0)	0 (0.0)	-

Data are expressed as *n* (%) or mean ± SD or unless otherwise specified. EBL: Estimated blood loss; LDP: Laparoscopic distal pancreatectomy; ODP: Open distal pancreatectomy.

Surgical outcomes in the LDP and ODP groups

Table 2 summarizes the operative outcomes and hospital courses of the LDP and ODP groups. There were no significant differences in the operating time (171 min *vs* 178 min, $P = 0.755$) between the two groups. LDP produced a significantly lower amount of intraoperative blood loss (149 mL *vs* 580 mL, $P = 0.002$), lower transfusion requirement (6.7% *vs* 46.2%, $P = 0.029$), shorter first flatus time (1.9 d *vs* 3.5 d, $P = 0.000$), shorter diet start time (2.3 d *vs* 4.9 d, $P = 0.000$), and shorter postoperative hospital stay (8.1 d *vs* 12.8 d, $P = 0.029$) than ODP.

There were no significant differences in postoperative complication rates (33.3% *vs* 38.5%, $P = 1.000$), pancreatic fistula rates (26.7% *vs* 30.8%, $P = 0.972$), and reoperation rates (0.0% *vs* 7.7%, $P = 0.464$) between the two groups. One patient underwent laparotomy for acute peritonitis after open spleen-preserving DP. We found biliary and pancreatic fistulas from the pancreatic stump. A calculus (diameter 6 mm) was incarcerated in the distal common bile duct, which led to bile regurgitation through the pancreaticobiliary common channel. The procedure consisted of cholecystectomy, common bile duct exploration, T tube drainage, and suture of the pancreatic remnant. The patient was discharged 24 d after the second operation. No perioperative mortality was recorded.

Pathological characteristics

Table 3 shows the pathological characteristics of the two groups. All patients had negative surgical margins at final

Table 3 Pathological characteristics of patients undergoing laparoscopic distal pancreatectomy or open distal pancreatectomy for pancreatic solid pseudopapillary tumor *n* (%)

Characteristics	LDP (*n* = 15)	ODP (*n* = 13)	*P* value
Harvested lymph nodes	4.6 ± 4.1	6.4 ± 6.2	0.549
Negative surgical margin,	15 (100.0)	13 (100.0)	-
Invasion of peripancreatic tissue	4 (26.7)	2 (15.4)	0.655
Perineural invasion	1 (6.7)	1 (7.7)	1.000
Liver metastasis	0 (0.0)	0 (0.0)	-
Lymphatic metastasis	0 (0.0)	0 (0.0)	-
Invasion of adjacent organs	0 (0.0)	0 (0.0)	-
Angioinvasion	0 (0.0)	0 (0.0)	-

Data are expressed as *n* (%) or mean ± SD or unless otherwise specified. LDP: Laparoscopic distal pancreatectomy; ODP: Open distal pancreatectomy.

pathology. An average number of 5.3 lymph nodes were resected without metastases. There was no significant difference in the number of harvested lymph nodes (4.6 *vs* 6.4, $P = 0.549$) between the two groups. In seven (25%) patients, the pathological findings were consistent with malignant features of SPT[14]. The malignant features included local invasion of peripancreatic tissue ($n = 6$), perineural invasion ($n = 2$), no liver metastasis, invasion of adjacent organs and angioinvasion. There were no significant differences in the pathological characteristics between the two groups.

Long-term outcomes

The median follow-up was 33 (3-100) mo for the LDP group and 45 (17-127) mo for the ODP group. All patients were alive with one recurrence. A 57-year-old female patient underwent ODP, and the pathology report revealed SPT with peripancreatic tissue invasion and perineural invasion. Six years after surgery, she developed peritoneal recurrence, which was treated by open tumorectomy and traditional Chinese medicine. At a follow-up of 15 mo after the second operation, no tumor recurrence was found. After surgery, six patients developed pancreatic exocrine or endocrine insufficiency; two received pancreatic enzyme therapy; and one developed diabetes and received insulin therapy. There were three cases of hyperglycemia with diet control.

DISCUSSION

SPT is an uncommon pancreatic neoplasm with nonspecific symptoms or completely asymptomatic[1]. A review of 718 patients with SPT showed that the most common localization of the tumor was the distal pancreas [tail (247 patients, 35.9%), body (102 patients, 14.8%), and body and tail (71 patients, 10.3%)][1]. This was also demonstrated in our series (29 patients, 52.7%). Therefore, DP with/without splenectomy is the most common surgical procedure for SPT. Complete resection of SPT offers benefits in almost all patients, and extensive lymphatic dissection is not indicated[1,15]. With the feasibility and safety of LDP being proven[16,17], it seems that LDP is thought to be more appropriate for SPT of the distal pancreas.

The first surgical resection of a pancreatic SPT was performed in 1970 and laparoscopic SPT resection in 2003[18,19]. The first series of laparoscopic SPT resection (10 cases) was published by Cavallini *et al*[20] in 2011. They regarded that LDP was a safe and feasible procedure for patients with SPT. However, no comparative analysis with open surgery was done. Kang *et al*[21] found smaller tumor size, earlier oral intake, and shorter hospital stay, without increasing morbidity in the laparoscopic (8 cases)/robot-assisted (3 cases) surgery group ($P < 0.05$) compared with open surgery group. To the best of our knowledge, the present series is the largest comparison of LDP and ODP for SPT. Our results indicated that LDP for SPT was associated with less operative blood loss and transfusion requirement, earlier first flatus and diet start, and shorter hospital stay compared to ODP, without increasing surgery-related risks (Table 2). The pathological examination showed that LDP for SPT provided similar oncological outcomes (harvested lymph nodes and margin status) as compared with ODP (Table 3). Long-term outcomes of laparoscopic surgery were comparable to those of open surgery. We believe that LDP for SPT could produce better short-term outcomes than ODP, without affecting oncological and long-term outcomes.

Our data and literature[1] showed that patients with SPT are expected to have a long-term survival after resection. At a median follow-up of 39 mo, 6 patients developed pancreatic exocrine or endocrine insufficiency. Thus, quality of life should be considered when choosing surgical procedure. Function-preserving laparoscopic pancreatectomy, including laparoscopic central pancreatectomy (LCP), spleen-preserving (SP)-LDP is thought to be an ideal procedure for this tumor. Some experts have reported the surgical technique of LCP with operative outcomes in small case series[9,10]. Three patients with SPT underwent LCP in our center. Nevertheless, the number of patients was too small to draw any conclusion. With the advances in instrumentation and accumulating experience, LCP would be an alternative procedure for SPT in the neck or proximal body of the pancreas.

As compared with SP-LDP, LDP with splenectomy tends to impair quality of life, with frequent higher-grade complications and prolonged hospital stays[22]. Butturini *et al*[23] compared the results of patients who underwent SP-LDP with or without splenic vessel conservation, and showed that postoperative morbidity did not differ between the two groups. The rate of perigastric varices was 60.0% after splenic vessel resection and 21.7% after splenic vessel conservation ($P = 0.123$)[23]. No gastrointestinal bleeding occurred at a median follow-up of 69 (37-139) mo[23]. In our series, only two patients underwent SP-LDP with splenic vessel conservation and 13 patients underwent LDP with splenectomy. For the small number of cases, there was no comparability between SP-LDP with splenic vessel conservation group and LDP with

splenectomy group. Considering the low malignancy of SPT and high rate of perigastric varices after splenic vessel resection, it is best to try to preserve the spleen with splenic vessels.

Recently, Fais *et al*[24] reported three patients with recurrences within 3 years after resection for SPT (laparoscopic biopsy with resection in one case, and laparoscopic biopsy and open resection in two cases). They considered that recurrence after laparoscopic biopsy may be due to diffusion of tumor cells caused by gas insufflation[24]. In our series, 15 patients underwent LDP without biopsy or broken specimen. At a median follow-up of 33 mo, all patients were alive without recurrence. In our opinion, laparoscopic biopsy should not be performed in patients with SPT. During laparoscopic surgery, we should make sure that the integrity of the specimen is not broken.

The limitations of this study were its retrospective design and low number of patients. These problems can be overcome only by a large, prospective randomized trial, which would be difficult to accomplish owning to the infrequent diagnosis of patients with SPT of the distal pancreas. We believe that this study could provide useful evidence in clinical practice.

In conclusion, LDP for SPT is feasible and safe, and has short-term benefits compared with ODP. Long-term outcomes are similar for LDP and ODP.

COMMENTS

Background

Solid pseudopapillary tumor (SPT) of the pancreas is a rare neoplasm. Some patients have benefited from laparoscopic pancreatectomy. Laparoscopic distal pancreatectomy (LDP) has not previously been compared with open distal pancreatectomy (ODP) for SPT.

Research frontiers

Recently, several case reports and small series have shown less intraoperative blood loss and rapid recovery after LDP for SPT. However, the short-term and long-term outcomes of LDP compared with ODP for SPT required further assessment.

Innovations and breakthroughs

In the present study, the authors compared the short-term and long-term outcomes of LDP and ODP for SPT, and showed that LDP was suitable and minimally invasive for treating SPT and could achieve similar oncological outcomes (harvested lymph nodes and margin status) and long-term outcomes as ODP.

Applications

This study showed that LDP for SPT had the advantages of minimally invasive surgery, less intraoperative blood loss, and rapid recovery. The mortality, morbidity, oncologic outcomes and long-term results of LDP were similar to those of ODP. These findings are helpful in decision-making for the treatment of SPT of the distal pancreas.

Peer review

The topic is interesting, and despite the rarity of SPT, the study includes a large series of patients.

REFERENCES

1 **Papavramidis T**, Papavramidis S. Solid pseudopapillary tumors of the pancreas: review of 718 patients reported in English literature. *J Am Coll Surg* 2005; **200**: 965-972 [PMID: 15922212 DOI: 10.1016/j.jamcollsurg.2005.02.011]

2 **Frantz VK**. Tumors of the pancreas. In: Atlas of tumor pathology. Washington DC: US Armed Forces Institute of Pathology, 1959: 32-33

3 **Mortenson MM**, Katz MH, Tamm EP, Bhutani MS, Wang H, Evans DB, Fleming JB. Current diagnosis and management of unusual pancreatic tumors. *Am J Surg* 2008; **196**: 100-113 [PMID: 18466869 DOI: 10.1016/j.amjsurg.2008.02.005]

4 **Klöppel G**, Solcia E, Longnecker DS, Capella C, Sobin LH. Histological typing of tumors of the exocrine pancreas. World Health Organization International Histological Classification of Tumours. 2nd ed. New York: Springer, 1996: 120-128

5 **Choi SH**, Kim SM, Oh JT, Park JY, Seo JM, Lee SK. Solid pseudopapillary tumor of the pancreas: a multicenter study of 23 pediatric cases. *J Pediatr Surg* 2006; **41**: 1992-1995 [PMID: 17161189 DOI: 10.1016/j.jpedsurg.2006.08.024]

6 **Dedieu A**, Rault A, Collet D, Masson B, Sa Cunha A. Laparoscopic enucleation of pancreatic neoplasm. *Surg Endosc* 2011; **25**: 572-576 [PMID: 20623235 DOI: 10.1007/s00464-010-1223-7]

7 **Venkat R**, Edil BH, Schulick RD, Lidor AO, Makary MA, Wolfgang CL. Laparoscopic distal pancreatectomy is associated with significantly less overall morbidity compared to the open technique: a systematic review and meta-analysis. *Ann Surg* 2012; **255**: 1048-1059 [PMID: 22511003 DOI: 10.1097/SLA.0b013e318251ee09]

8 **Asbun HJ**, Stauffer JA. Laparoscopic vs open pancreaticoduodenectomy: overall outcomes and severity of complications using the Accordion Severity Grading System. *J Am Coll Surg* 2012; **215**: 810-819 [PMID: 22999327 DOI: 10.1016/j.jamcollsurg.2012.08.006]

9 **Sa Cunha A**, Rault A, Beau C, Collet D, Masson B. Laparoscopic central pancreatectomy: single institution experience of 6 patients. *Surgery* 2007; **142**: 405-409 [PMID: 17723894 DOI: 10.1016/j.surg.2007.01.035]

10 **Rotellar F**, Pardo F, Montiel C, Benito A, Regueira FM, Poveda I, Martí-Cruchaga P, Cienfuegos JA. Totally laparoscopic Roux-en-Y duct-to-mucosa pancreaticojejunostomy after middle pancreatectomy: a consecutive nine-case series at a single institution. *Ann Surg* 2008; **247**: 938-944 [PMID: 18520220 DOI: 10.1097/SLA.0b013e3181724e4a]

11 **Yan JF**, Mou YP, Xu XW, Ni JJ, Chen DW, Zhu YP, Chen QL, Zhou YC, Xie K. [Laparoscopic distal pancreatectomy: the experience of 68 cases in a single centre]. *Zhonghua Waike Zazhi* 2012; **50**: 802-805 [PMID: 23157954 DOI: 10.3760/cma.j.issn.0529-5815.2012.09.008]

12 **Xu XW**, Li RH, Zhou W, Wang J, Zhang RC, Chen K, Mou YP. Laparoscopic resection of synchronous intraductal papillary mucinous neoplasms: a case report. *World J Gastroenterol* 2012; **18**: 6510-6514 [PMID: 23197900 DOI: 10.3748/wjg.v18.i44.6510]

13 **Bassi C**, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery* 2005; **138**: 8-13 [PMID: 16003309 DOI: 10.1016/j.surg.2005.05.001]

14 **Klöppel G**, Lüttges J, Klimstra D, Hruban R, Kern S, Adler G. Solid-pseudopapillary neoplasm. In: Hamilton SR, Aaltonene LA, editors. World Health Organization classification of tumors: pathology and genetics of tumors of the digestive system. Lyon (France): IARC Press, 2000: 246-248

15 **Yoon DY**, Hines OJ, Bilchik AJ, Lewin K, Cortina G, Reber HA. Solid and papillary epithelial neoplasms of the pancreas: aggressive resection for cure. *Am Surg* 2001; **67**: 1195-1199 [PMID: 11768829]

16 **Baker MS**, Bentrem DJ, Ujiki MB, Stocker S, Talamonti MS. A prospective single institution comparison of peri-operative outcomes for laparoscopic and open distal pancreatectomy. *Surgery* 2009; **146**: 635-43; discussion 643-5 [PMID: 19789022 DOI: 10.1016/j.surg.2009.06.045]

17 **Kooby DA**, Gillespie T, Bentrem D, Nakeeb A, Schmidt MC, Merchant NB, Parikh AA, Martin RC, Scoggins CR, Ahmad S, Kim HJ, Park J, Johnston F, Strouch MJ, Menze A,

Rymer J, McClaine R, Strasberg SM, Talamonti MS, Staley CA, McMasters KM, Lowy AM, Byrd-Sellers J, Wood WC, Hawkins WG. Left-sided pancreatectomy: a multicenter comparison of laparoscopic and open approaches. *Ann Surg* 2008; **248**: 438-446 [PMID: 18791364 DOI: 10.1097/SLA.0b013e318185a990]

18 **Hamoudi AB**, Misugi K, Grosfeld JL, Reiner CB. Papillary epithelial neoplasm of pancreas in a child. Report of a case with electron microscopy. *Cancer* 1970; **26**: 1126-1134 [PMID: 5476792 DOI: 10.1002/1097-0142(197011)]

19 **Carricaburu E**, Enezian G, Bonnard A, Berrebi D, Belarbi N, Huot O, Aigrain Y, de Lagausie P. Laparoscopic distal pancreatectomy for Frantz's tumor in a child. *Surg Endosc* 2003; **17**: 2028-2031 [PMID: 14598158 DOI: 10.1007/s00464-003-4222-0]

20 **Cavallini A**, Butturini G, Daskalaki D, Salvia R, Melotti G, Piccoli M, Bassi C, Pederzoli P. Laparoscopic pancreatectomy for solid pseudo-papillary tumors of the pancreas is a suitable technique; our experience with long-term follow-up and review of the literature. *Ann Surg Oncol* 2011; **18**: 352-357 [PMID: 20848223 DOI: 10.1245/s10434-010-1332-5]

21 **Kang CM**, Choi SH, Hwang HK, Lee WJ, Chi HS. Minimally invasive (laparoscopic and robot-assisted) approach for solid pseudopapillary tumor of the distal pancreas: a single-center experience. *J Hepatobiliary Pancreat Sci* 2011; **18**: 87-93 [PMID: 20676697 DOI: 10.1007/s00534-010-0316-y]

22 **Choi SH**, Seo MA, Hwang HK, Kang CM, Lee WJ. Is it worthwhile to preserve adult spleen in laparoscopic distal pancreatectomy? Perioperative and patient-reported outcome analysis. *Surg Endosc* 2012; **26**: 3149-3156 [PMID: 22580876 DOI: 10.1007/s00464-012-2306-4]

23 **Butturini G**, Inama M, Malleo G, Manfredi R, Melotti GL, Piccoli M, Perandini S, Pederzoli P, Bassi C. Perioperative and long-term results of laparoscopic spleen-preserving distal pancreatectomy with or without splenic vessels conservation: a retrospective analysis. *J Surg Oncol* 2012; **105**: 387-392 [PMID: 22025322 DOI: 10.1002/jso.22117]

24 **Fais PO**, Carricaburu E, Sarnacki S, Berrebi D, Orbach D, Baudoin V, de Lagausie P. Is laparoscopic management suitable for solid pseudo-papillary tumors of the pancreas? *Pediatr Surg Int* 2009; **25**: 617-621 [PMID: 19479267 DOI: 10.1007/s00383-009-2388-9]

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BRIEF ARTICLE

De novo combined lamivudine and adefovir dipivoxil therapy *vs* entecavir monotherapy for hepatitis B virus-related decompensated cirrhosis

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Abstract

AIM: To compare efficacy of combined lamivudine (LAM) and adefovir dipivoxil (ADV) therapy with that of entecavir (ETV) monotherapy for hepatitis B virus (HBV)-related decompensated liver cirrhosis.

METHODS: A total of 120 naïve patients with HBV-related decompensated cirrhosis participated in this study. Sixty patients were treated with combined LAM and ADV therapy (LAM + ADV group), while the other 60 were treated with ETV monotherapy (ETV group) for two years. Tests for liver and kidney function, alpha-fetoprotein, HBV serum markers, HBV DNA load, prothrombin time (PT), and ultrasonography or computed tomography scan of the liver were performed every 1 to 3 mo. Repeated measure ANOVA and the χ^2 test were performed to compare the efficacy, side effects, and the cumulative survival rates at 48 and 96 wk.

RESULTS: Forty-five patients in each group were observed for 96 wk. No significant differences in HBV DNA negative rates and alanine aminotransferase (ALT) normalization rates at weeks 48 (χ^2 = 2.12 and 2.88) and 96 (χ^2 = 3.21 and 3.24) between the two groups were observed. Hepatitis B e antigen seroconversion rate in the LAM + ADV group at week 96 was significantly higher in the ETV group (43.5% *vs* 36.4%, χ^2 = 4.09, P < 0.05). Viral breakthrough occurred in 2 cases (4.4%) by week 48 and in 3 cases (6.7%) by week 96 in the LAM + ADV group, and no viral mutation was detected. In the ETV group, viral breakthrough occurred in 1 case (2.2%) at the end of week 96. An increase in albumin (F = 18.9 and 17.3), decrease in total bilirubin and in ALT (F = 16.5, 17.1 and 23.7, 24.8), reduced PT (F = 22.7 and 24.5), and improved Child-Turcotte-Pugh and the model for end-stage liver disease scores (F = 18.5, 17.8, and 24.2, 23.8) were observed in both groups. The cumulative rates of mortality and liver transplantation were 16.7% (10/60) and 18.3% (11/60) in the LAM + ADV and ETV groups, respectively.

CONCLUSION: Both LAM + ADV combination therapy and ETV monotherapy can effectively inhibit HBV replication, improve liver function, and decrease mortality.

Key words: Chronic hepatitis B; Decompensated liver cirrhosis; Lamivudine; Adefovir dipivoxil; Combination therapy; Entecavir

Core tip: This study compared the *de novo* efficacy of combined lamivudine (LAM) and adefovir dipivoxil (ADV) therapy with that of entecavir (ETV) monotherapy for patients with hepatitis B virus (HBV)-related decompensated liver cirrhosis. Both LAM + ADV combination therapy and ETV monotherapy can effectively inhibit HBV replication, improve liver function, and decrease mortality. The data obtained in this study demonstrate the efficacy and the safety of these treatment regimens for 96 wk in patients with HBV-related decompensated liver cirrhosis.

Lian JS, Zeng LY, Chen JY, Jia HY, Zhang YM, Xiang DR, Yu L, Hu JH, Lu YF, Zheng L, Li LJ, Yang YD. *De novo* combined lamivudine and adefovir dipivoxil therapy *vs* entecavir monotherapy for hepatitis B virus-related decompensated cirrhosis. *World J Gastroenterol* 2013; 19(37): 6278-6283 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6278.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6278

INTRODUCTION

Cirrhosis is the end stage of chronic liver damage and is characterized by fibrosis resulting in the distortion and destruction of normal liver architecture. Functional liver tissue is destroyed and replaced by regenerating nodules that do not fully restore lost liver function. Cirrhosis may be due to various causes, including hepatitis B virus (HBV) infection, hepatitis C (HCV) and alcohol consumption[1,2]. Chronic infection with HBV accounts for 30% of hepatic cirrhosis globally[3]. In China, about 93 million people are carriers of HBV, with 20 million people chronically infected. Within a five-year period, 10% to 20% of patients with chronic hepatitis B develop cirrhosis[4]. The five-year survival rates of patients with compensated cirrhosis and of those with decompensated cirrhosis (determined by the presence of ascites, hepatoencephalopathy, and/or history of variceal bleeding) were 84% and 14%, respectively[5]. Cirrhosis precedes most cases of hepatocellular carcinoma (HCC), with 70% to 90% of HCC developing from liver cirrhosis or inflammation[6]. Antiviral agents are assumed to reduce decompensated cirrhosis and HCC development[7], however, agents such as lamivudine (LAM) and telbivudin show high drug resistance. The latest chronic hepatitis B prevention and treatment guidelines suggest the selection of a higher genetic barrier to resistant antivirals, such as entecavir (ETV) and tenofovir, for patients with HBV-related liver cirrhosis[8,9]. However, based on the paradigm that drug combination therapy is more effective than monotherapy for the treatment of human immunodeficiency virus and HCV, the same approach may be appropriate for chronic hepatitis B. This study was designed to compare the two-year efficacy of *de novo* combination therapy of LAM and adefovir dipivoxil (ADV) with that of ETV monotherapy in patients with decompensated liver cirrhosis.

MATERIALS AND METHODS

Study patients

From January 2008 to March 2009, 120 patients diagnosed with HBV-related decompensated liver cirrhosis at the First Affiliated Hospital of the Zhejiang University School of Medicine (Hangzhou, China) were recruited into this study. The diagnosis was based on medical history, the results of physical examination, biochemical, endoscopic and ultrasound findings, and radiological signs of cirrhosis. All patients were 18 to 65 years old, with $\geqslant 10^3$ copies/mL HBV DNA, 7 to 12 (inclusive) Child-Turcotte-Pugh (CTP) score, $\geqslant$ 50 mL calculated serum creatinine clearance, $\geqslant$ 75 g/L hemoglobin, $\geqslant 2.5 \times 10^9$/L total white blood cells, $\leqslant$ 20 ng/mL α-fetoprotein, and no evidence of HCC. None of the patients had been treated with antiviral drugs, including interferon-α or nucleos(t)ides. Patients with hepatitis delta virus, hepatitis C virus, or had human immunodeficiency virus (HIV) co-infection were excluded. Patients with HCC, autoimmune hepatitis, alcoholic liver cirrhosis, hepatorenal syndrome, grade 3 or 4 hepatic encephalopathy, spontaneous bacterial peritonitis, and severe heart, renal, and brain diseases were also excluded. All patients who participated in this study provided informed consent and were aware of the procedures to be conducted. The protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University.

Study design

The study was designed as a prospective case-control study. The patients were randomly assigned to the ETV monotherapy (60 patients) group and the *de novo* LAM and ADV combination therapy (60 patients) group. Baseline data of the two groups were compared to ensure comparability. Patients in the combination therapy group were prescribed 100 mg LAM and 10 mg ADV per day, while the monotherapy group received 0.5 mg ETV per day.

Follow-up studies

Serum hepatitis B viral markers, including hepatitis B surface antigen (HBsAg), antibody to HBsAg, hepatitis B e antigen (HBeAg), antibody to HBeAg and antibody to hepatitis B core antigen, were detected by commercially available enzyme immunoassays (Abbott Laboratories; Chicago, IL, United States). Serum HBV DNA was measured by polymerase chain reaction with a linear range between 1×10^3 and 5×10^8 copies/mL (Shanghai ZJ Bio-Tech Co., Ltd., China).

Follow-up observations in the two groups were performed at the start and during weeks 4, 12, 24, 36, 48, 60, 72, 84, and 96. Follow-up clinical assessments included physical examination, HBeAg and antibodies to the e antigen, quantitative HBV DNA, serum biochemistry, alpha-fetoprotein, renal function, prothrombin time (PT), and ultrasonography or computed tomography scan. The lower limit of detection of DNA used in this study was 1.0×10^3 copies/mL (Shanghai ZJ Bio-Tec Co., Ltd, Chi-

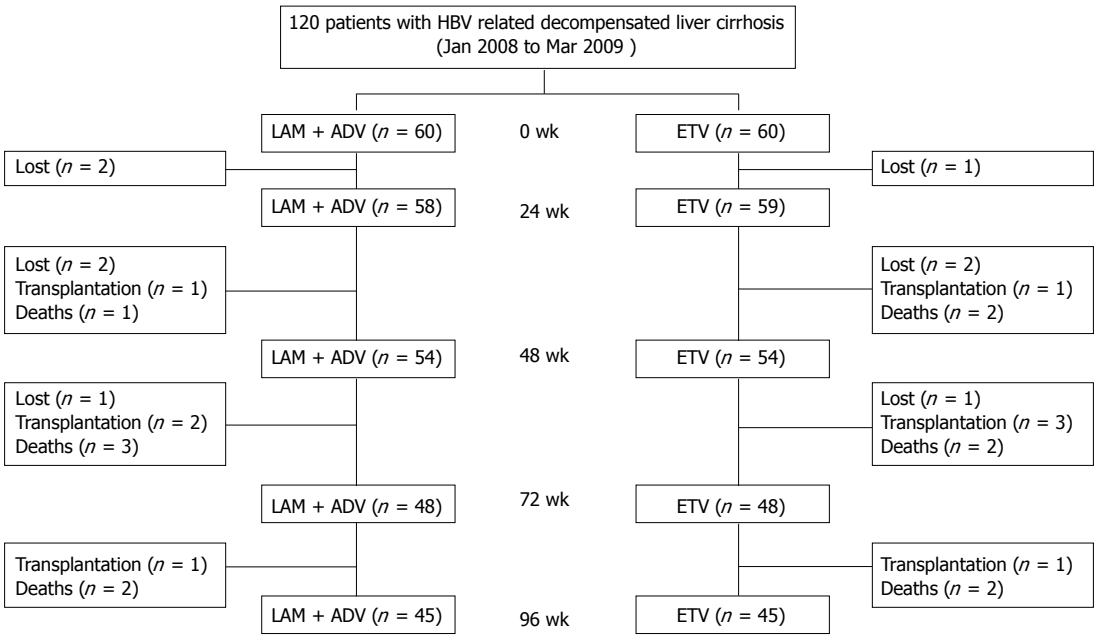

Figure 1 Flow chart of patient conditions following treatment with *de novo* lamivudine and adefovir dipivoxil combination therapy and entecavir monotherapy for 96 wk. LAM: Lamivudine; ADV: Adefovir dipivoxil; ETV: Entecavir.

na). The condition of the patients after 96 wk is shown in Figure 1.

Statistical analysis

SPSS 16.0 software was used for data analysis. Measurements were presented as mean ± SD and comparisons were conducted following analysis of the results using the Student's *t* test. Proportions were presented as percentage (%). Rate comparisons were performed using the χ^2 test. A P value < 0.05 was considered significant.

RESULTS

Baseline characteristics

In the two years of follow-up observations, of the 60 patients who received LAM and ADV combination therapy, 5 cases were lost, 4 cases underwent liver transplantation, 6 cases died, and 45 cases survived until the end of the observation period. The 45 remaining cases comprised 16 females and 29 males. The mean patient age was 53.1 ± 8.8 years. Of the 60 patients who received ETV monotherapy, 4 cases were lost, 5 cases underwent liver transplantation, 6 cases died, and 45 cases survived until the end of the observation period. The 45 remaining cases comprised 17 females and 28 males. The mean patient age was 53.2 ± 7.4 years. The baseline characteristics of the patients were similar, and no statistically significant differences were observed (Table 1).

Virological, serological and biochemical response

Of the 45 patients in the LAM and ADV combination group, 51.1% (23/45) and 86.7% (39/45) achieved undetectable HBV DNA by weeks 48 and 96, respectively. Of the 45 patients in the ETV group, 60% (27/45) and 88.9% (40/45) achieved undetectable HBV DNA by weeks 48 and 96, respectively. No statistical differences were observed between the two groups by weeks 48 and 96 ($P > 0.05$).

In the LAM and ADV combination therapy group, 71.1% (32/45) and 88.9% (40/45) of patients achieved ALT normalization by week 48 and 96, respectively. In the ETV treatment group, 68.9% (31/45) and 91.1% (41/45) achieved ALT normalization by week 48 and 96, respectively. No statistical difference was observed between the two groups at week 48 and 96 ($P > 0.05$).

Of the 45 patients who received the LAM and ADV combination treatment, 30.4% (7/23) and 43.5% (10/23) achieved HBeAg seroconversion by weeks 48 and 96, respectively. Similarly, 27.3% (6/22) and 36.4% (8/22) of the patients who received ETV monotherapy achieved HBeAg seroconversion by weeks 48 and 96, respectively. No statistical difference was observed between the two groups at week 48, while the HBeAg seroconversion rate in the LAM and ADV combination group at week 96 was significantly higher than that in the ETV monotherapy group (43.5% *vs* 36.4%, $\chi^2 = 4.09$, $P < 0.05$).

Of the respondents, 2 and 3 patients in the LAM and ADV combination group and 1 and 2 patients in the ETV monotherapy group developed virological breakthrough by weeks 48 and 96, respectively. No genetic mutations were detected in either patient group. The obtained differences were not statistically different ($P > 0.05$).

Changes in liver function in patients with decompensated cirrhosis

After 96 wk of treatment, the albumin level in patients in the LAM and ADV combination group increased sig-

Table 1 Baseline characteristics of patients with HBV-related decompensated cirrhosis *n* (%)

Variables	LAM + ADV (*n* = 45)	ETV (*n* = 45)	*t*	χ^2	*P* value
Age (yr)	53.1 ± 8.8	53.2 ± 7.4	0.23		> 0.05
Male/female	29/16	28/17		3.10	> 0.05
HBV DNA (log10 copy/mL)	6.56 ± 1.13	6.61 ± 1.15	0.33		> 0.05
ALT (U/L)	98.1 ± 21.6	99.8 ± 17.2	0.23		> 0.05
TBil (μmol/L)	51.6 ± 8.9	49.2 ± 6.8	0.31		> 0.05
Alb (g/L)	29.2 ± 0.7	29.5 ± 1.2	0.24		> 0.05
PT (s)	16.3 ± 2.3	16.5 ± 1.9	0.21		> 0.05
CTP score	8.4 ± 1.7	8.6 ± 2.1	0.16		> 0.05
MELD score	13.7 ± 3.5	12.9 ± 6.7	0.25		> 0.05
HBeAg positive rate	23 (51.1)	22 (48.9)		2.13	> 0.05
Ascites	22 (48.9)	21 (46.7)		2.46	> 0.05
HE	6 (13.3)	7 (15.5)		3.13	> 0.05
UGB	9 (22.2)	8 (17.8)		3.35	> 0.05

ALT: Alanine aminotransferase; TBil: Total bilirubin; Alb: Albumin; PT: Prothrombin time: CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; HE: Hepatic encephalopathy; UGB: Upper gastrointestinal bleeding; HBeAg: Hepatitis B e antigen.

nificantly compared with the baseline level (F = 18.9, P < 0.05), whereas ALT and TBil decreased significantly compared with the baseline levels (F = 16.5 and 23.7, respectively, P < 0.05). In addition, PT was significantly shortened (F = 22.7, P < 0.05), and both CTP and MELD scores decreased significantly compared with the baseline scores (F = 18.5 and 24.2, respectively, P < 0.05). A decrease of more than 2 points in the CTP score in 31 (68.9%) cases was observed and is shown in Table 2.

After 96 wk, patients who received ETV treatment exhibited a significant increase in albumin level compared with the baseline level (F = 17.3, P < 0.05). In contrast, ALT and TBil decreased significantly compared with baseline levels (F = 17.1 and 24.8, P < 0.05). PT was significantly shortened (F = 24.5, P < 0.05), and CTP and MELD scores decreased significantly compared with the baseline levels (F = 17.8 and 23.8, P < 0.05). A decrease in the CTP score by more than 2 points was evident in 30 (66.7%) cases.

The LAM and ADV combination group and the ETV monotherapy group showed no significant differences in albumin level or in ALT, TBil, PT, CTP, and MELD scores by weeks 48 and 96 (Table 2).

Adverse events

All patients in this study responded well to both LAM and ADV combination therapy and ETV monotherapy. Creatinine levels in four cases in the LAM and ADV combination therapy group and in one case in the ETV monotherapy group were more than twice the baseline values, but were still lower than upper limit of normal. No patient developed lactic acidosis in either group.

In the combination group, the cumulative mortality (including liver transplantation) was 16.7% (10/60) during the follow-up period, and included 2 cases of upper gastrointestinal bleeding, 2 cases of hepatic encephalopathy, 1 case of secondary bacterial infection, and 1 case of hepatorenal syndrome. Four patients had undergone liver transplantation in this group. In the ETV monotherapy group, the cumulative mortality (including liver transplantation) was 18.3% (11/60), and included 3 cases of upper gastrointestinal bleeding, 2 cases of secondary bacterial infection, and 2 cases of hepatorenal syndrome. Three patients had undergone liver transplantation. These findings are illustrated in Figure 2.

DISCUSSION

Increasing evidence shows that suppression of HBV replication results in the reduction of hepatic necroinflammation and consequently, improvement of liver function in patients with HBV-related decompensated liver cirrhosis. Antiviral therapy associated with improved outcomes in patients with HBV-related decompensated cirrhosis, including postponement or prevention of liver transplantation, reducing the incidence of HCC[10-12].

LAM was the first oral agent approved for the treatment of chronic hepatitis B (CHB) and currently has a well-established safety and efficacy profile. Liaw *et al*[13] reported that continuous treatment with LAM delays clinical progression of CHB infection in patients by significantly reducing the incidence of hepatic decompensation and HCC. ADV benefits pre- and post-transplant patients with LAM-resistant CHB, including decompensated cirrhotics, by suppressing HBV DNA and by improving the CTP score[14,15]. In China, ADV is relatively cheap and a large number of CHB patients, including cirrhotics, have received LAM and ADV combination therapy. According to the latest guidelines, the patients with liver cirrhosis and those who have received a liver graft for HBV-related cirrhosis should be considered for *de novo* combination therapy because of the risk of clinical deterioration if they develop drug resistance[16]. But the data to support a role of combination therapy in these patients were limited. On the other hand, ETV demonstrates very low rates of resistance in nucleoside-naïve patients and is recommended for patients with HBV-related decompensated cirrhosis[8,17]. Therefore, a comparison of the efficacy and safety between LAM and ADV combination therapy and ETV monotherapy for patients with HBV-related decompensated cirrhosis is urgent. Our study showed that 51.1% and 86.7% of patients in the LAM and ADV combination group achieved undetectable HBV DNA by weeks 48 and 96, respectively, while 60% and 88.9% of patients in the ETV treatment group achieved undetectable HBV DNA by weeks 48 and 96, respectively. In addition, both *de novo* combination of LAM and ADV therapy and ETV monotherapy significantly increased albumin level and decreased TBil, PT, CTP, and MELD scores compared with baseline. More importantly, 68.9% of patients in the combination group and 66.7% of patients in the monotherapy group had a decrease in their CTP score of more than 2 points after 96 wk of treatment. A total of 73.7% of patients in the combination group and 71.1% of patients in the monotherapy group exhibited an increase in the CTP score at the end of 96

Table 2 Comparison of changes in hepatic function

Characteristics	LAM + ADV combination group			ETV monotherapy group		
	0 wk	48 wk	96 wk	0 wk	48 wk	96 wk
Alb (g/L)	29.2 ± 0.7	32.2 ± 0.5	36.7 ± 0.2[a]	28.9 ± 1.2	31.9 ± 0.4	36.4 ± 0.6[a]
TBil (μmol/L)	51.6 ± 8.9	30.8 ± 7.5	19.1 ± 6.2[a]	47.2 ± 6.8	31.6 ± 6.8	18.2 ± 3.9[a]
ALT (U/L)	98.1 ± 21.6	56.1 ± 21.3	34.7 ± 12.8[a]	99.8 ± 17.2	54.2 ± 15.7	32.5 ± 11.5[a]
PT (s)	16.3 ± 2.3	14.3 ± 1.6	12.6 ± 2.1[a]	16.5 ± 1.9	13.8 ± 2.0	12.9 ± 3.7[a]
CTP score	8.4 ± 1.7	6.8 ± 1.9	5.5 ± 3.7[a]	8.6 ± 2.1	6.7 ± 2.5	5.7 ± 1.3[a]
MELD score	13.7 ± 3.5	9.8 ± 3.1	7.6 ± 1.8[a]	12.9 ± 6.7	9.6 ± 4.3	7.9 ± 2.3[a]

[a]$P < 0.05$ *vs* baseline. ALT: Alanine aminotransferase; TBil: Total bilirubin; Alb: Albumin; PT: Prothrombin time: CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; LAM: Lamivudine; ADV: Adefovir dipivoxil; ETV: Entecavir.

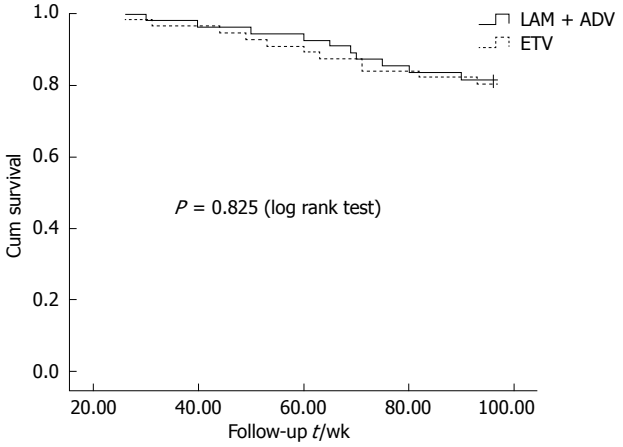

Figure 2 Kaplan-Meier analysis of cumulative survival rate in patients with hepatitis B virus-related decompensated cirrhosis treated with lamivudine and adefovir dipivoxil combination therapy and entecavir monotherapy for 96 wk. LAM: Lamivudine; ADV: Adefovir dipivoxil; ETV: Entecavir.

wk. No genetic mutations in either treatment group were detected. In this study, no statistically significant difference was observed between the LAM and ADV combination therapy group and the ETV monotherapy group in terms of the serological conversion rate by 48 wk, while the HBeAg seroconversion rate in the LAM and ADV combination group at week 96 was significantly higher than that in the ETV group. This finding is similar to the results of previous studies[18].

HBV-related decompensated cirrhosis requires a longer duration of antiviral therapy and consideration of the effect and safety of these drugs are essential. LAM has been shown to be safe. ADV, in contrast, is mainly excreted by the kidney and has an impact on renal function during long-term antiviral therapy[19]. Our study confirms that ADV treatment of decompensated cirrhosis is safe and effective. However, in the subsequent stages of treatment, doctors should closely monitor kidney function and adjust the treatment plan as soon as renal function is found to be abnormal.

The best treatment method for late-stage HBV-related decompensated liver cirrhosis is liver transplantation. However, transplantation is very expensive and there is a worldwide donor shortage. Liver transplantation is considered in the treatment of decompensated cirrhosis only when the CTP score for grade C or the MELD score is more than 20 points. Upon detection of HBV DNA, patients with decompensated cirrhosis should be immediately treated with antiviral therapy to improve liver function and to reduce the need for liver transplantation.

Lange *et al*[20] reported on 16 patients with liver cirrhosis and chronic hepatitis B who were treated with ETV. Five of these patients developed lactic acidosis (all with MELD scores > 20) during ETV treatment. Lactic acidosis was lethal for one patient, while for other patients, the symptoms were resolved after termination/interruption of ETV treatment. In the present study, no cases of lactic acidosis were observed during the follow-up period of 96 wk.

In conclusion, both *de novo* LAM and ADV combination therapy and ETV monotherapy are effective in patients with HBV-related decompensated cirrhosis, with no differences in the level of HBV DNA suppression, liver function improvement, resistance rate, and on confirmed changes in renal parameters and in cumulative survival rate. The data obtained in this study demonstrate the efficacy and the safety of these treatment regimens for 96 wk in patients with HBV-related decompensated liver cirrhosis, as well as their evident therapeutic benefits in both groups.

COMMENTS

Background

The mortality rate of hepatitis B virus (HBV)-related decompensated cirrhosis is very high. Recommended treatment options are monotherapy with high genetic barrier nucleos(t)ide analogues or combination therapy with no cross resistance nucleos(t)ide analogues. There has been no report regarding the entecavir monotherapy or *de novo* lamivudine and adefovir dipivoxil combination therapy in these patients.

Research frontiers

De novo combination therapy with lamivudine and adefovir dipivoxil is better than lamivudine monotherapy in patients with HBV-related decompensated live cirrhosis. But there is no head to head research to compare the entecavir, a high genetic barrier nucleoside analogue monotherapy with *de novo* lamivudine and adefovir dipivoxil combination therapy for those patients. In this study, the authors demonstrated that both entecavir monotherapy and *de novo* lamivudine and adefovir dipivoxil combination therapy were effective for patients with HBV-related decompensated liver cirrhosis.

Innovations and breakthroughs

Many clinical studies showed that the combined therapy is effective for patients with human immunodeficiency virus and hepatitis C virus infection. And entecavir is effective for patients with HBV-related decompensated liver cirrhosis.

This is the first head to head study to report that both *de novo* lamivudine and adefovir dipivoxil combination therapy and entecavir monotherapy are effective for patients with HBV-related decompensated liver cirrhosis.

Applications

By understanding that both *de novo* lamivudine and adefovir dipivoxil combination therapy and entecavir monotherapy are effective for patients with HBV-related decompensated liver cirrhosis, this study may represent a future strategy for therapeutic intervention in patients with HBV-related decompensated liver cirrhosis.

Terminology

De novo combination therapy means combination with two or more drugs from the beginning of the treatment. Monotherapy means use one drug form the beginning of the treatment. The diagnosis of decompensated liver cirrhosis was based on clinical, laboratory, previous histological, ultrasonographic and radiological signs of cirrhosis with Child-Turcotte-Pugh (CTP) score. The CTP score is a system to assess the disease stage for decompensated cirrhotic patients.

Peer review

This is a good clinical study in which the authors compared the effects of *de novo* lamivdine and adefovir dipivoxil combination therapy with entecavir monotherapy for HBV-related decompensated liver cirrhosis patients. The authors concluded that both *de novo* lamivudine and adefovir dipivoxil combination therapy and entecavir monotherapy are effective for patients with HBV-related decompensated liver cirrhosis.

REFERENCES

1 **Myers RP**, Shaheen AA, Hubbard JN, Kaplan GG. Characteristics of patients with cirrhosis who are discharged from the hospital against medical advice. *Clin Gastroenterol Hepatol* 2009; **7**: 786-792 [PMID: 19345284 DOI: 10.1016/j.cgh.2009.03.020]

2 **Lefton HB**, Rosa A, Cohen M. Diagnosis and epidemiology of cirrhosis. *Med Clin North Am* 2009; **93**: 787-799, vii [PMID: 19577114 DOI: 10.1016/j.mcna.2009.03.002]

3 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]

4 **Liu J**, Fan D. Hepatitis B in China. *Lancet* 2007; **369**: 1582-1583 [PMID: 17499584 DOI: 10.1016/S0140-6736(07)60723-5]

5 **de Jongh FE**, Janssen HL, de Man RA, Hop WC, Schalm SW, van Blankenstein M. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. *Gastroenterology* 1992; **103**: 1630-1635 [PMID: 1426884]

6 **Schütte K**, Bornschein J, Malfertheiner P. Hepatocellular carcinoma--epidemiological trends and risk factors. *Dig Dis* 2009; **27**: 80-92 [PMID: 19546545 DOI: 10.1159/000218339]

7 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531 [PMID: 15470215 DOI: 10.1056/NEJMoa033364]

8 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]

9 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283 [PMID: 19669255 DOI: 10.1007/s12072-008-9080-3]

10 **Guan R**, Lui HF. Treatment of hepatitis B in decompensated liver cirrhosis. *Int J Hepatol* 2011; **2011**: 918017 [PMID: 21994876 DOI: 10.4061/2011/918017]

11 **Fontana RJ**. Management of patients with decompensated HBV cirrhosis. *Semin Liver Dis* 2003; **23**: 89-100 [PMID: 12616454 DOI: 10.1055/s-2003-37591]

12 **Fontana RJ**, Hann HW, Perrillo RP, Vierling JM, Wright T, Rakela J, Anschuetz G, Davis R, Gardner SD, Brown NA. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. *Gastroenterology* 2002; **123**: 719-727 [PMID: 12198698]

13 **Liaw YF**, Raptopoulou-Gigi M, Cheinquer H, Sarin SK, Tanwandee T, Leung N, Peng CY, Myers RP, Brown RS, Jeffers L, Tsai N, Bialkowska J, Tang S, Beebe S, Cooney E. Efficacy and safety of entecavir versus adefovir in chronic hepatitis B patients with hepatic decompensation: a randomized, open-label study. *Hepatology* 2011; **54**: 91-100 [PMID: 21503940 DOI: 10.1002/hep.24361]

14 **Schiff ER**, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann HL, Samuel D, Zeuzem S, Lilly L, Rendina M, Villeneuve JP, Lama N, James C, Wulfsohn MS, Namini H, Westland C, Xiong S, Choy GS, Van Doren S, Fry J, Brosgart CL. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post-liver transplantation patients. *Hepatology* 2003; **38**: 1419-1427 [PMID: 14647053 DOI: 10.1016/j.hep.2003.09.040]

15 **Schiff E**, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann H, Samuel D, Zeuzem S, Villeneuve JP, Arterburn S, Borroto-Esoda K, Brosgart C, Chuck S. Adefovir dipivoxil for wait-listed and post-liver transplantation patients with lamivudine-resistant hepatitis B: final long-term results. *Liver Transpl* 2007; **13**: 349-360 [PMID: 17326221 DOI: 10.1002/lt.20981]

16 **Carey I**, Harrison PM. Monotherapy versus combination therapy for the treatment of chronic hepatitis B. *Expert Opin Investig Drugs* 2009; **18**: 1655-1666 [PMID: 19852566 DOI: 10.1517/13543780903241599]

17 **Singal AK**, Fontana RJ. Meta-analysis: oral anti-viral agents in adults with decompensated hepatitis B virus cirrhosis. *Aliment Pharmacol Ther* 2012; **35**: 674-689 [PMID: 22257108 DOI: 10.1111/j.1365-2036.2011.04990.x]

18 **Chang TT**, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001-1010 [PMID: 16525137 DOI: 10.1056/NEJMoa051285]

19 **Viganò M**, Lampertico P, Colombo M. Drug safety evaluation of adefovir in HBV infection. *Expert Opin Drug Saf* 2011; **10**: 809-818 [PMID: 21671843 DOI: 10.1517/14740338.2011.593507]

20 **Lange CM**, Bojunga J, Hofmann WP, Wunder K, Mihm U, Zeuzem S, Sarrazin C. Severe lactic acidosis during treatment of chronic hepatitis B with entecavir in patients with impaired liver function. *Hepatology* 2009; **50**: 2001-2006 [PMID: 19937695 DOI: 10.1002/hep.23346]

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META-ANALYSIS

Endoscopic ultrasound elastography for differentiating between pancreatic adenocarcinoma and inflammatory masses: A meta-analysis

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Abstract

AIM: To evaluate the accuracy of endoscopic ultrasound (EUS) elastography for differentiating between pancreatic ductal adenocarcinoma (PDAC) and pancreatic inflammatory masses (PIM).

METHODS: Electronic databases (updated to December 2012) and manual bibliographical searches were carried out. A meta-analysis of all diagnostic clinical trials evaluating the accuracy of EUS elastography in differentiating PDAC from PIM was conducted. Heterogeneity was assessed among the studies. The meta-analysis was performed to evaluate the accuracy of EUS elastography in differentiating PDAC from PIM in homogeneous studies.

RESULTS: Ten studies involving 781 patients were included in the analysis. Significant heterogeneity in sensitivity was observed among the studies (Cochran Q test = 24.16, df = 9, P = 0.0041, I^2 = 62.8%), while heterogeneity in specificity was not observed (Cochran Q test = 5.93, df = 9, P = 0.7473, I^2 = 0.0%). The area under the curve under the Sports Rights Owners Coalition was 0.8227. Evaluation of heterogeneity suggested that the different diagnostic standards used in the included studies were the source of heterogeneity. In studies using the color pattern as the diagnostic standard, the pooled sensitivity, specificity, positive likelihood ratio (LR), negative LR and diagnostic OR were 0.99 (0.97-1.00), 0.76 (0.67-0.83), 3.36 (2.39-4.72), 0.03 (0.01-0.07) and 129.96 (47.02-359.16), respectively. In studies using the hue histogram as the diagnostic standard, the pooled sensitivity, specificity, positive LR, negative LR and diagnostic OR were 0.92 (0.89-0.95), 0.68 (0.57-0.78), 2.84 (2.05-3.93), 0.12 (0.08-0.19) and 24.69 (12.81-47.59), respectively.

CONCLUSION: EUS elastography is a valuable method for the differential diagnosis between PDAC and PIM. And a preferable diagnostic standard should be explored and improvements in specificity are required.

Key words: Endoscopic ultrasound; Elastography; Pancreatic adenocarcinoma; Meta-analysis

Core tip: Pancreatic inflammatory masses (PIM) are easily confused with pancreatic ductal adenocarcinoma (PDAC). Endoscopic ultrasound (EUS) elastography is a promising noninvasive method for differentiating between PDAC and PIM and may prove to be a valuable supplemental method to EUS-guided fine-needle aspiration.

Li X, Xu W, Shi J, Lin Y, Zeng X. Endoscopic ultrasound elastography for differentiating between pancreatic adenocarcinoma and

inflammatory masses: A meta-analysis. *World J Gastroenterol* 2013; 19(37): 6284-6291 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6284.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6284

INTRODUCTION

Pancreatic cancer is a highly lethal disease, and approximately 90% of pancreatic tumors are pancreatic ductal adenocarcinoma (PDAC) which has an extremely poor prognosis[1,2]. The 5-year survival rate of PDAC is as low as 0.2%[3]. The only potentially curable treatment which is surgical resection, relies on early diagnosis[4]. Pancreatic inflammatory masses (PIM) are confused with PDAC[5]. The differential diagnosis between PDAC and PIM is currently still difficult due to non-specific symptoms, signs or imaging presentations[6].

Endoscopic ultrasound (EUS) elastography is a recently developed technique for the differential diagnosis of benign and malignant pancreatic masses and measures the mechanical properties of tissues[7-14]. The tissue elasticity modulus is represented by a transparent color superimposed on the conventional gray-scale B-mode scans. The nature of the tissue is analyzed either by a qualitative method where blue-predominant represents malignancy or a quantitative method where a value of more than 175 represents malignancy.

Pancreatic masses include PDAC, PIM, neuroendocrine tumors, metastatic tumors, lymphoma, sarcoma, insulinoma and lipoma. Several meta-analyses have evaluated the accuracy of EUS elastography in the diagnosis of pancreatic masses. The overall accuracy of EUS elastography in differentiating between PDAC and PIM has not been assessed. The aim of this study was to perform a meta-analysis of existing studies to assess the accuracy of EUS elastography in differentiating between PDAC and PIM.

MATERIALS AND METHODS

Study selection

Studies were selected according to the inclusion and exclusion criteria which were delineated prior to the literature search. The inclusion criteria were: (1) diagnostic clinical trials assessing the accuracy of EUS elastography for differentiating between PDAC and PIM; (2) cytology of EUS-guided fine-needle aspiration (FNA) samples, histopathology of surgical specimens or a follow-up period of at least 6 months as a reference standard; and (3) sufficient data to construct a 2 × 2 table for true-positive, false-positive, false-negative and true-negative findings.

Studies were excluded if they met the following criteria: (1) studies without complete data available for constructing a 2 × 2 table for true-positive, false-positive, false-negative and true-negative findings; (2) studies updated or duplicated; (3) studies which did not report their own data such as editorials, reviews, corresponding letters; and (4) case reports.

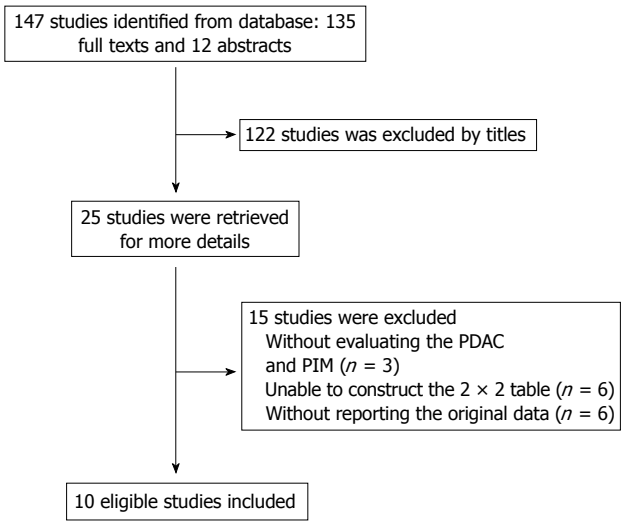

Figure 1 Literature search flow diagram.

Literature search

Using the Medline, Embase, Web of Science, and Cochrane Central Trials databases up to Dec. 2012, a systematic literature search was conducted. The search strategy was ("elastogram" or "elastography" or "elastosonoendoscopy" or "sonoelastography") and ("pancreatic" or "pancreas" or "adenocarcinoma" or "inflammatory mass"). To expand the search, we also performed a manual search of abstracts presented at the United European Gastroenterology Week (UEGW) congresses and the American Digestive Disease Week (DDW) from 2000 to 2012. The bibliographies of each peer-reviewed paper were screened for other potentially relevant studies. If missing data were needed, we contacted the appropriate authors by mail.

Statistical analysis

Data on the differentiation between PDAC and PIM were extracted. The Cochrane Q test was used to assess heterogeneity with a P value < 0.10[15]. I^2 was used to describe the percentage variability attributable to heterogeneity rather than sampling errors. I^2 > 25% indicated the presence of heterogeneity. The Spearman ϱ between the logit of sensitivity and logit of 1-specificity was calculated to assess the presence of a threshold effect. A strong correlation (Spearman ϱ < -0.4) suggested the presence of a threshold effect[16]. The source of heterogeneity, with the exception of the threshold effect, was explored by meta-regression analysis[17,18]. The subgroups were predefined, and included diagnostic standard (color pattern *vs* hue histogram), blind (yes *vs* unclear), sample size (≥ 50 *vs* < 50), type of publication (full text *vs* abstract), and design of study (single center *vs* multicenter). A P value < 0.05 indicated significance. Pooling was only

Table 1 Baseline characteristics of the studies in the analysis

Ref.	Type of publication	Design of study	Diagnostic standard	Cut-off	No.
Săftoiu *et al*[7]	Full text	Single center	Hue histogram	> 175	54
Iglesias-Garcia *et al*[8]	Full text	Single center	Color pattern	Blue-predominant	76
Janssen *et al*[9]	Full text	Single center	Color pattern	Blue-predominant	25
Deprez *et al*[10]	Abstract	Single center	Color pattern	Blue-predominant	13
Săftoiu *et al*[11]	Full text	Single center	Hue histogram	> 175	43
Iglesias-Garcia *et al*[12]	Full text	Single center	Color pattern	Blue-predominant	119
Giovannini *et al*[13]	Full text	Multicenter	Color pattern	Blue-predominant	96
Giovannini *et al*[14]	Full text	Single center	Color pattern	Blue-predominant	18
Itokawa *et al*[24]	Full text	Single center	Color pattern	Blue-predominant	79
Săftoiu *et al*[34]	Full text	Multicenter	Color pattern	> 175	258

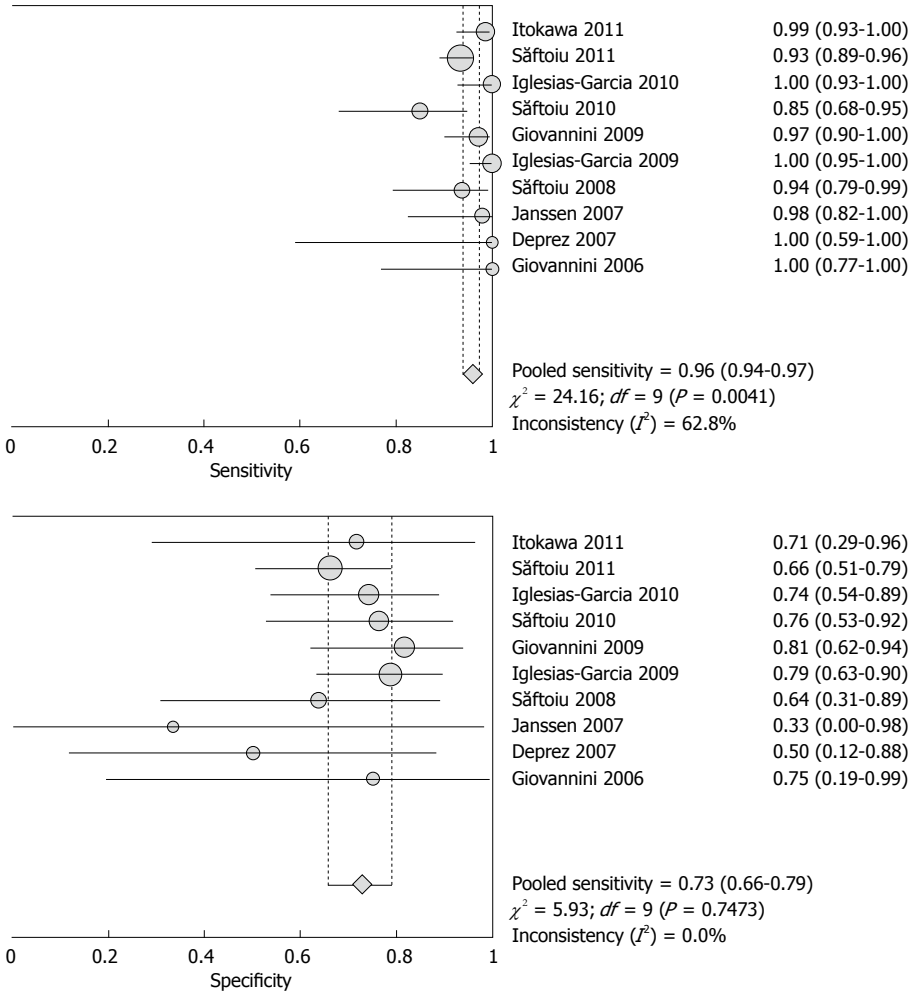

Figure 2 Forest plot (random-effect model) of the meta-analysis for sensitivity (upper) and specificity (lower) in differentiating between pancreatic ductal adenocarcinoma and pancreatic inflammatory masses.

conducted within the homogeneous groups using the fixed-effect model (Mantel-Haenszel method[19]). Pooling the results with corresponding 95%CI included sensitivity, specificity, positive likelihood ratio (LR), negative LR and diagnostic odds ratio (DOR).

In order to analyze the presence of publication bias, funnel plots were constructed using the Harbord[20] and Egger indicator and Begg[21] and Mazumdar indicator. Asymmetric funnel plots or a P value < 0.1 suggested the presence of publication bias. The quality of the selected studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) questionnaire[22]. Items were rated as yes, no, or unclear.

The pooled weighted sensitivity, specificity, positive LR, negative LR, DOR, Sports Rights Owners Coalition (SROC) curve and Spearman analysis were performed using Meta-Disc version 1.4 (Unit of Clinical Biostatistics, Ramony Cajal Hospital, Madrid, Spain)[23]. Meta-regression and publication bias analyses were performed using Stata version 10.0 (Stata Corporation, College Sta-

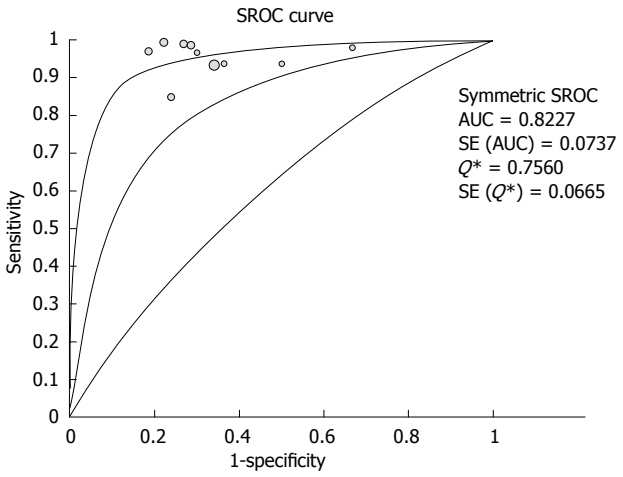

Figure 3 Sports Rights Owners Coalition, with 95%CI, for differentiating between pancreatic ductal adenocarcinoma and pancreatic inflammatory masses.

tion, TX, United States).

RESULTS

The initial literature search identified a total of 147 studies (Figure 1). Of these 147 studies, 25 potentially relevant studies were retrieved for further evaluation. Ten studies involving 781 patients were finally included in this meta-analysis. The baseline characteristics of the selected studies are listed in Table 1. Nine studies were published as full texts, and 1 as an abstract. Seven studies used the color pattern as the diagnostic standard, while the other three used the hue histogram value.

Differentiating PDAC and PIM

The pooled sensitivity and specificity (random-effect model) of EUS elastography for differentiating between PDAC and PIM were 96% (95%CI: 94-97) and 73% (95%CI: 66-79), respectively. Significant heterogeneity in sensitivity was observed among the studies (Cochran Q test = 24.16, df = 9, P = 0.0041, I^2 = 62.8%), while heterogeneity in specificity was not observed (Cochran Q test = 5.93, df = 9, P = 0.7473, I^2 = 0.0%) (Figure 2). The AUC under the SROC was 0.8227 (Figure 3).

By excluding the study reported as an abstract, the pooled sensitivity and specificity (random-effect model) were 96% (95%CI: 94-97) and 73% (95%CI: 66-80), respectively. There was significant heterogeneity in sensitivity among the studies (Cochran Q test =23.56, df = 8, P = 0.0027, I^2 = 66.1%), while heterogeneity in specificity was not observed (Cochran Q test = 5.50, df = 8, P = 0.8090, I^2 = 0.0%). The AUC under the SROC was 0.8188.

Test of heterogeneity

The source of heterogeneity was explored. A Spearman ρ of -0.29 (P = 0.41) between the logit of sensitivity and the logit of 1-specificity did not suggest the presence of a threshold effect. The meta-regression analysis showed that the different diagnostic standards used in the selected studies were the source of heterogeneity (P = 0.00). In addition, the characteristics of blinding, sample size, type of publication and design of study were not related to heterogeneity (Table 2).

Table 2 Meta-regression analysis for the potential source of heterogeneity

Study characteristics	*Z*	*P* value	95%CI
Diagnostic standard (color pattern *vs* hue histogram)	2.90	0.00	0.68-3.50
Blind (yes *vs* unclear)	1.36	0.17	-0.87-4.82
Sample size (≥ 50 *vs* < 50)	0.13	0.90	-1.90-2.17
Type of publication (full text *vs* abstract)	1.28	0.20	-1.33-6.37
Design of study (single center *vs* multicenter)	0.04	0.97	-1.35-1.40

Table 3 Subgroup analysis on the basis of the diagnostic standards

Pooled estimate	Color pattern (n = 426)[1]		Hue histogram (n = 355)[2]	
	Pooled result (95%CI)	I^2	Pooled result (95%CI)	I^2
Sensitivity	0.99 (0.97-1.00)	0.00%	0.92 (0.89-0.95)	20.10%
Specificity	0.76 (0.67-0.83)	0.00%	0.68 (0.57-0.78)	0.00%
Positive LR	3.36 (2.39-4.72)	17.90%	2.84 (2.05-3.93)	0.00%
Negative LR	0.03 (0.01-0.07)	0.00%	0.12 (0.08-0.19)	0.00%
Diagnostic OR	129.96 (47.02-359.16)	0.00%	24.69 (12.81-47.59)	0.00%

[1]Studies using the color pattern as the diagnostic standard and the total number of patients involved; [2]Studies using the hue histogram as the diagnostic standard and the total number of patients involved. OR: Odds ratio; LR: Likelihood ratio.

Meta-analysis based on diagnostic standards

The evaluation of heterogeneity suggested that the different diagnostic standards used in the included studies were the source of heterogeneity. As a result, the meta-analysis was performed on the studies using the same diagnostic standards. The pooled results showed good homogeneity. Pooling was conducted using the fixed-effect model (Mantel-Haenszel method[22]). In studies using the color pattern as the diagnostic standard, the pooled sensitivity, specificity, positive LR, negative LR and DOR were 0.99 (0.97-1.00), 0.76 (0.67-0.83), 3.36 (2.39-4.72), 0.03 (0.01-0.07) and 129.96 (47.02-359.16), respectively. In studies using the hue histogram as the diagnostic standard, the pooled sensitivity, specificity, positive LR, negative LR and DOR were 0.92 (0.89-0.95), 0.68 (0.57-0.78), 2.84 (2.05-3.93), 0.12 (0.08-0.19) and 24.69 (12.81-47.59), respectively (Table 3).

Quality assessment using the QUADAS questionnaire

The quality of the selected studies according to the QUADAS questionnaire is shown in Figure 4. The overall quality of the studies was good. Eight studies were rated as "yes" in all items. In the study by Janssen *et al*[9]

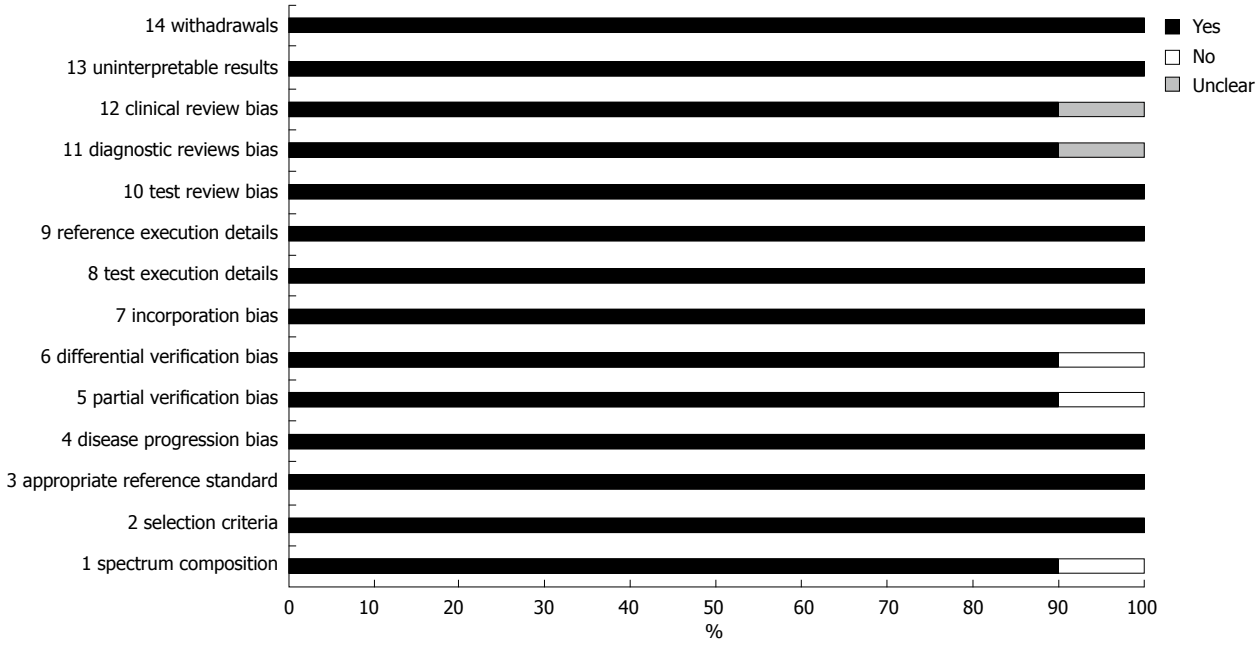

Figure 4 The Quality Assessment of Diagnostic Accuracy Studies scores of the selected studies are summed up per item and presented in a bar chart.

all selected patients were referred for EUS-guided FNA or surgery and one of the selected patients was diagnosed with lipoma by CT densitometry without histological proof. As a result, QUADAS question 1, 5 and 6 were rated as "no". In addition, Jassen *et al*[9] and Itokawa *et al*[24] did not mention whether blinding was used in their study. As a result, QUADAS question 11 was rated as "unclear".

Publication bias

The Harbord-Egger indicator for publication bias provided a value of 1.65 (95%CI: -0.43-2.59, $P = 0.14$) and the Begg-Mazumdar indicator gave a Kendall's tau *b* value of 9 ($P = 0.47$) for the selected studies, which suggested no publication bias (Figure 5).

DISCUSSION

Pancreatic cancer is the fourth leading cause of cancer-related death in the USA, and the second among gastrointestinal tumors[25]. Early diagnosis may allow patients to receive the only potentially curable treatment which is surgical resection. PDAC is found in more than 90% of patients with pancreatic cancer and most of the lesions confused with PDAC are benign PIM[5]. PDAC is frequently associated with secondary inflammatory changes caused by obstruction of the pancreatic duct. In addition, chronic pancreatitis can markedly increase the risk of PDAC[26]. As a result, the differential diagnosis between PDAC and PIM is essential for clinical decision-making.

Despite considerable advances in imaging techniques, the diagnosis of PDAC, particularly in the setting of chronic pancreatitis, remains a challenge. There are no characteristic findings to differentiate pancreatic masses on transabdominal ultrasound (TAS) and its accuracy is very low[27]. Computed tomography (CT) and magnetic resonance imaging (MRI) may be used for staging and detecting metastasis, however, these techniques have limited ability in differentiating between PDAC and PIM[28,29]. Endoscopic retrograde cholangiopancreatography (ERCP) has an increased risk of complications, the most important being pancreatitis[30].

EUS, which provides high-resolution images of the pancreas, has become an indispensable tool in the management of pancreatic diseases. However, an important limitation of EUS examination is its low capacity to determine the exact nature of pancreatic masses[31]. EUS-FNA allows pathological diagnosis. It is currently considered an accurate and safe method for the diagnosis of pancreatic disease. However, EUS-FNA is an invasive procedure and the sensitivity of EUS-FNA is less than 75% in the presence of coexistent chronic pancreatitis or "pseudotumoral" pancreatitis[32,33].

EUS elastography is a newly developed technique which assesses the mechanical properties of tissues during conventional EUS examination. In this meta-analysis, no significant publication bias was detected using the Harbord-Egger and Begg-Mazumdar indicators. The meta-regression analysis demonstrated that the different diagnostic standards used in the included studies may be the source of heterogeneity. This meta-analysis indicated that EUS elastography could achieve a very high sensitivity and a moderate specificity for differentiating between PDAC and PIM. As EUS elastography showed good sensitivity it may be an appropriate method for monitoring patients with PIM in whom malignancy has been excluded. In addition, it could also be used to fol-

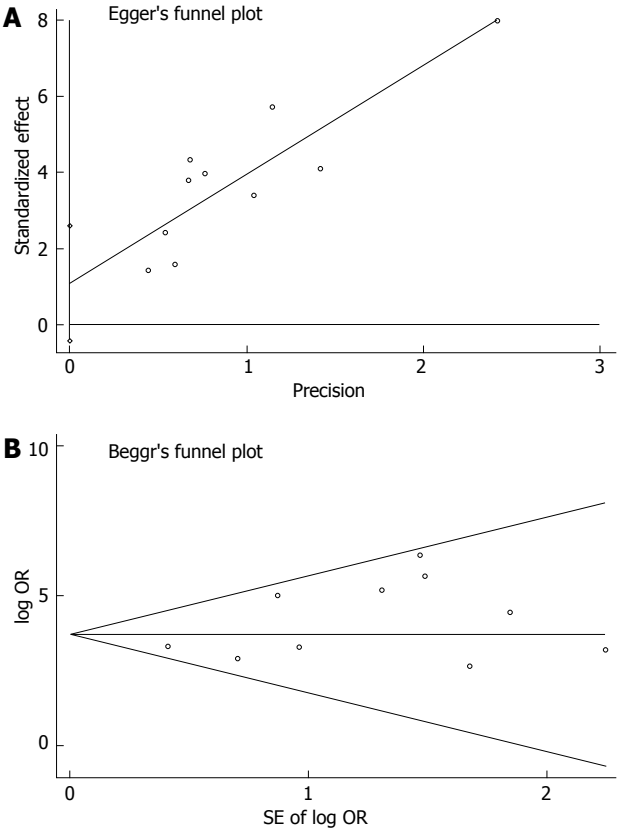

Figure 5 Funnel plots. A: Funnel plot of the Harbord-Egger indicator for the selected studies; B: Funnel plot of the Begg-Mazumdar indicator for the selected studies.

low patients with PDAC after surgery.

The pooled specificity of EUS elastography may not be satisfactory for differentiating between PDAC and PIM compared with the 100% specificity of EUS-FNA. This may be due to the following reasons: first, diagnostic studies preferred maximal sensitivity in order to reduce the false negative rate when setting up the cutoff value. This reduced the specificity of individual studies. Second, this study focused on the differentiation between PDAC and PIM. The data from normal controls and chronic pancreatitis patients without focal masses, which could easily be excluded from malignancy by EUS elastography, were excluded from this study. This would markedly reduce the number of true negative cases, and thereby decrease specificity.

As an imaging method with moderate specificity, EUS elastography could not replace EUS-FNA which provides a pathological diagnosis. However, it may be a valuable supplemental method to EUS-FNA. EUS elastography and FNA could be performed sequentially during the same EUS procedure. It could be used to guide FNA to reduce the number of false negative cases, especially in patients with coexisting pancreatitis. Moreover, EUS elastography may provide additional information for differentiating between PDAC and PIM when a negative EUS-FNA result is obtained or the patients are unsuitable for FNA.

The diagnostic standard used for the analysis of mechanical properties was correlated with the accuracy of EUS elastography in the differentiation of pancreatic masses. The qualitative color pattern and quantitative hue histogram value are two currently used diagnostic standards. In general, the quantitative diagnostic standard would be considered better because it is an objective method. Base on unified samples (PDAC and PIM), a subgroup analysis was performed to compare these two standards. The results showed that studies using the color pattern as the diagnostic standard showed preferable pooled estimates than those using the hue histogram. This may be due to the fact that both the overall stiffness and the distribution of stiffness were associated with the nature of the tissue. The color pattern diagnostic standard takes the predominant color and the distribution of the color into consideration simultaneously, while the hue histogram value only gives overall stiffness.

There were some limitations in this meta-analysis. One of the selected studies was published as an abstract, and some details were not available. A small number of studies were included in this study which may have reduced the power of the analysis.

In conclusion, EUS elastography is a valuable method for the differential diagnosis between PDAC and PIM. And a preferable diagnostic standard should be explored and improvements in specificity are required.

COMMENTS

Background

Endoscopic ultrasound (EUS) elastography is a recently developed technique for the differential diagnosis of benign and malignant pancreatic masses and measures the mechanical properties of tissues. The overall accuracy of EUS elastography in differentiating between pancreatic ductal adenocarcinoma (PDAC) and pancreatic inflammatory masses (PIM) has not been assessed.

Research frontiers

Several meta-analyses on the accuracy of EUS elastography in the diagnosis of pancreatic masses have been carried out. The overall accuracy of EUS elastography in differentiating between PDAC and PIM has not been assessed.

Innovations and breakthroughs

Previous studies have mainly focused on the differential diagnosis of benign and malignant pancreatic masses. This analysis suggested that EUS elastography could achieve a very high sensitivity and a moderate specificity for differentiating PDAC from PIM. Such findings were not presented clearly in previous studies.

Applications

This analysis suggested that EUS elastography could achieve a very high sensitivity and a moderate specificity for differentiating PDAC from PIM. Due to good sensitivity, EUS elastography may be an appropriate method for monitoring patients with PIM in whom malignancy has been excluded. In addition, it could be used to follow patients with PDAC after surgery.

Peer review

This is a well-performed meta-analysis of currently available studies on the accuracy of EUS elastography in the differential diagnosis between PDAC and PIM. The authors found that EUS elastography is a promising noninvasive method for differential diagnosis of PDAC and PIM and may prove to be a valuable supplemental method to EUS-guided fine-needle aspiration. This is a good meta-analysis and the authors have included many relevant issues missed by other research groups.

REFERENCES

1 **Gudjonsson B**. Survival statistics gone awry: pancreatic cancer, a case in point. *J Clin Gastroenterol* 2002; **35**: 180-184 [PMID: 12172365 DOI: 10.1097/00004836-200208000-00011]
2 **Gudjonsson B**. Cancer of the pancreas. 50 years of surgery. *Cancer* 1987; **60**: 2284-2303 [PMID: 3326653]
3 **Carpelan-Holmström M**, Nordling S, Pukkala E, Sankila R, Lüttges J, Klöppel G, Haglund C. Does anyone survive pancreatic ductal adenocarcinoma? A nationwide study re-evaluating the data of the Finnish Cancer Registry. *Gut* 2005; **54**: 385-387 [PMID: 15710987 DOI: 10.1136/gut.2004.047191]
4 **Trede M**, Schwall G, Saeger HD. Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. *Ann Surg* 1990; **211**: 447-458 [PMID: 2322039 DOI: 10.1097/00000658-199004000-00011]
5 **van Gulik TM**, Reeders JW, Bosma A, Moojen TM, Smits NJ, Allema JH, Rauws EA, Offerhaus GJ, Obertop H, Gouma DJ. Incidence and clinical findings of benign, inflammatory disease in patients resected for presumed pancreatic head cancer. *Gastrointest Endosc* 1997; **46**: 417-423 [PMID: 9402115 DOI: 10.1016/S0016-5107(97)70034-8]
6 **Niederau C**, Grendell JH. Diagnosis of pancreatic carcinoma. Imaging techniques and tumor markers. *Pancreas* 1992; **7**: 66-86 [PMID: 1557348 DOI: 10.1097/00006676-199201000-00011]
7 **Săftoiu A**, Iordache SA, Gheonea DI, Popescu C, Maloş A, Gorunescu F, Ciurea T, Iordache A, Popescu GL, Manea CT. Combined contrast-enhanced power Doppler and real-time sonoelastography performed during EUS, used in the differential diagnosis of focal pancreatic masses (with videos). *Gastrointest Endosc* 2010; **72**: 739-747 [PMID: 20674916 DOI: 10.1016/j.gie.2010.02.056]
8 **Iglesias-Garcia J**, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. *Gastroenterology* 2010; **139**: 1172-1180 [PMID: 20600020 DOI: 10.1053/j.gastro.2010.06.059]
9 **Janssen J**, Schlörer E, Greiner L. EUS elastography of the pancreas: feasibility and pattern description of the normal pancreas, chronic pancreatitis, and focal pancreatic lesions. *Gastrointest Endosc* 2007; **65**: 971-978 [PMID: 17531630 DOI: 10.1016/j.gie.2006.12.057]
10 **Deprez PH**, Yeung CPR, Weynand B, Gillain C, Gigot JF, Hubert C, Horsmans Y, Borbath I. Contrast EUS Versus EUS Sono-Elastography in the Differentiation of Atypical Pancreatic Masses. *Gastrointest Endosc* 2007; **65**: AB103 [DOI: 10.1016/j.gie.2007.03.075]
11 **Săftoiu A**, Vilmann P, Gorunescu F, Gheonea DI, Gorunescu M, Ciurea T, Popescu GL, Iordache A, Hassan H, Iordache S. Neural network analysis of dynamic sequences of EUS elastography used for the differential diagnosis of chronic pancreatitis and pancreatic cancer. *Gastrointest Endosc* 2008; **68**: 1086-1094 [PMID: 18656186 DOI: 10.1016/j.gie.2008.04.031]
12 **Iglesias-Garcia J**, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. EUS elastography for the characterization of solid pancreatic masses. *Gastrointest Endosc* 2009; **70**: 1101-1108 [PMID: 19647248 DOI: 10.1016/j.gie.2009.05.011]
13 **Giovannini M**, Thomas B, Erwan B, Christian P, Fabrice C, Benjamin E, Geneviève M, Paolo A, Pierre D, Robert Y, Walter S, Hanz S, Carl S, Christoph D, Pierre E, Jean-Luc VL, Jacques D, Peter V, Andrian S. Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: a multicenter study. *World J Gastroenterol* 2009; **15**: 1587-1593 [PMID: 19340900 DOI: 10.3748/wjg.15.1587]
14 **Giovannini M**, Hookey LC, Bories E, Pesenti C, Monges G, Delpero JR. Endoscopic ultrasound elastography: the first step towards virtual biopsy? Preliminary results in 49 patients. *Endoscopy* 2006; **38**: 344-348 [PMID: 16680632 DOI: 10.1055/s-2006-925158]
15 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]
16 **Devillé WL**, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; **2**: 9 [PMID: 12097142 DOI: 10.1186/1471-2288-2-9]
17 **Lijmer JG**, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. *Stat Med* 2002; **21**: 1525-1537 [PMID: 12111918 DOI: 10.1002/sim.1185]
18 **Glas AS**, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003; **56**: 1129-1135 [PMID: 14615004 DOI: 10.1016/S0895-4356(03)00177-X]
19 **Mantel N**, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; **22**: 719-748 [PMID: 13655060]
20 **Harbord RM**, Egger M, Sterne JA. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Stat Med* 2006; **25**: 3443-3457 [PMID: 16345038 DOI: 10.1002/sim.2380]
21 **Begg CB**, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101 [PMID: 7786990 DOI: 10.2307/253344]
22 **Whiting P**, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003; **3**: 25 [PMID: 14606960 DOI: 10.1186/1471-2288-3-25]
23 **Zamora J**, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. *BMC Med Res Methodol* 2006; **6**: 31 [PMID: 16836745 DOI: 10.1186/1471-2288-6-31]
24 **Itokawa F**, Itoi T, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Umeda J, Tanaka R, Yokoyama N, Moriyasu F, Kasuya K, Nagao T, Kamisawa T, Tsuchida A. EUS elastography combined with the strain ratio of tissue elasticity for diagnosis of solid pancreatic masses. *J Gastroenterol* 2011; **46**: 843-853 [PMID: 21505859 DOI: 10.1007/s00535-011-0399-5]
25 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]
26 **Malka D**, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, Lévy P, Ruszniewski P. Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut* 2002; **51**: 849-852 [PMID: 12427788 DOI: 10.1136/gut.51.6.849]
27 **Hessel SJ**, Siegelman SS, McNeil BJ, Sanders R, Adams DF, Alderson PO, Finberg HJ, Abrams HL. A prospective evaluation of computed tomography and ultrasound of the pancreas. *Radiology* 1982; **143**: 129-133 [PMID: 7063714]
28 **Rösch T**, Braig C, Gain T, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography. Comparison with conventional sonography, computed tomography, and angiography. *Gastroenterology* 1992; **102**: 188-199 [PMID: 1727753]
29 **Semelka RC**, Ascher SM. MR imaging of the pancreas. *Radiology* 1993; **188**: 593-602 [PMID: 8351317]
30 **Loperfido S**, Angelini G, Benedetti G, Chilovi F, Costan F, De Berardinis F, De Bernardin M, Ederle A, Fina P, Fratton A. Major early complications from diagnostic and therapeutic ERCP: a prospective multicenter study. *Gastrointest Endosc* 1998; **48**: 1-10 [PMID: 9684657 DOI: 10.1016/S0016-

5107(98)70121-X]
31 **Brand B**, Pfaff T, Binmoeller KF, Sriram PV, Fritscher-Ravens A, Knöfel WT, Jäckle S, Soehendra N. Endoscopic ultrasound for differential diagnosis of focal pancreatic lesions, confirmed by surgery. *Scand J Gastroenterol* 2000; **35**: 1221-1228 [PMID: 11145297 DOI: 10.1080/003655200750056736]
32 **Varadarajulu S**, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. *Gastrointest Endosc* 2005; **62**: 728-36; quiz 751, 753 [PMID: 16246688]
33 **Ardengh JC**, Lopes CV, Campos AD, Pereira de Lima LF, Venco F, Módena JL. Endoscopic ultrasound and fine needle aspiration in chronic pancreatitis: differential diagnosis between pseudotumoral masses and pancreatic cancer. *JOP* 2007; **8**: 413-421 [PMID: 17625292]
34 **Săftoiu A**, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, Iglesias-Garcia J, Arcidiacono P, Will U, Giovannini M, Dietrich C, Havre R, Gheorghe C, McKay C, Gheonea DI, Ciurea T. Accuracy of endoscopic ultrasound elastography used for differential diagnosis of focal pancreatic masses: a multicenter study. *Endoscopy* 2011; **43**: 596-603 [PMID: 21437851]

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CASE REPORT

Video capsule endoscopy and CT enterography in diagnosing adult hypertrophic pyloric stenosis

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Abstract

Primary adult hypertrophic pyloric stenosis is a rare but important cause of gastric outlet obstruction that may be misdiagnosed as idiopathic gastroparesis. Clinically, patients present with early satiety, abdominal fullness, nausea, epigastric discomfort and eructation. Permanent gastric retention of a video capsule endoscope is diagnostic in differentiating between the two diseases, in the absence of an organic gastric outlet obstruction. This case presents the longest video capsule retention in the medical literature to date. It is also the first case report of adult hypertrophic pyloric stenosis diagnosed with video capsule endoscopy or a computed tomography scan. Finally, an unusual "plugging" of the gastric outlet with free floating capsule has an augmented effect on disease physiology and on patient's symptoms.

Key words: Video capsule endoscopy; Hypertrophic pyloric stenosis; Gastroparesis; Endoscopy; Computed tomography enterography

Core tip: Classic descriptors and latest developments and potential role for capsule endoscopy in differential diagnosis of adult hypertrophic pyloric stenosis (HSP). First ever case of 3 years of video capsule retention in a patient. First ever report of diagnosing adult HPS with video capsule and/or with computed tomography-enterography. Physiologic effects of video capsule on symptoms in adult HPS. Differentiating between adult HPS and gastroparesis. Advances in treatment of adult HPS.

Gurvits GE, Tan A, Volkov D. Video capsule endoscopy and CT enterography in diagnosing adult hypertrophic pyloric stenosis. *World J Gastroenterol* 2013; 19(37): 6292-6295 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6292.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6292

INTRODUCTION

Video capsule endoscopy in an important tool in our ever growing arsenal in detecting bowel related abnormalities. Its wide use has lead to the significant improvement of our ability to understand and diagnose a variety of gastrointestinal (GI) diseases. Retention of the capsule is one of the feared complications of the procedure, however, its location can often point to the identifiable pathology in a patient with on obscure GI condition. In this case report, we present a truly unique situation in which a discovery of a retained capsule lead to the correct diagnosis of adult hypertrophic pyloric stenosis in a patient previously diagnosed with idiopathic gastroparesis. Interestingly, freely floating video capsule intermittently "plugged" already compromised gastric outlet, resulting in worsening of the patient's symptoms.

CASE REPORT

A 53-year-old female presented to our office for a second opinion evaluation of a progressive history of early satiety, weight loss, and postprandial abdominal fullness of over 10-year duration, with notable exacerbation of her symptoms over last 3 years. Her symptoms would typically worsen toward the evening. Her past medical history included depression, surgically excised breast cancer, gastroesophageal reflux disease, and eradication of *Helicobacter pylori* in the absence of peptic ulcer disease. Several prior esophagogastroduodenoscopies performed at different clinics were notable for persistent retention of solid food in the stomach despite overnight fasts and nuclear studies showed prolonged gastric emptying. The patient was diagnosed with gastroparesis, however, she failed to respond to trials of metoclopramide, domperidone, or erythromycin. Part of her previous work up also included a video capsule endoscopy in 2008 that commented on delayed gastric transit and an erroneous conclusion of a capsule entering small bowel after 7 h. On our initial physical exam, the patient appeared in no distress. Abdominal evaluation was unremarkable, including absence of a succession splash. Laboratory values were all within normal limits. A scout abdominal roentogram revealed a retained capsule in the gastric antrum, and a barium upper GI series demonstrated pronounced delay in gastric emptying with a narrowed slightly elongated pylorus and a distended gastric antrum (Figure 1A, B). Computed tomography (CT) enterography of the abdomen verified retained video capsule in the distended antrum of the stomach and visualized abnormally dense, eccentric, and significantly narrowed pylorus measuring 15 mm in thickness and 24 mm in length (Figure 1C). Endoscopic findings were remarkable for retained semi-digested food particles despite a two day liquid diet, and confirmed the presence of a 3-year-old video capsule freely floating in the gastric fundus. A notably fixed and narrow (7 mm pre-instrumentation) thickened pyloric channel was successfully traversed with a standard 10 mm endoscope applying moderate pressure (Figure 2). The pyloric mucosa appeared unremarkable with no mass lesion or ulceration. Evaluation of the proximal duodenum was unremarkable. Four quadrant biopsies of the pyloric channel did not reveal malignancy. The video capsule was endoscopically retrieved with a Roth Net®. The patient was diagnosed with adult hypertrophic pyloric stenosis that accounted for her symptoms. She refused possible endoscopic interventions with balloon dilation or Botox injection, and declined a surgical pyloroplasty. During follow-up at three and 6 mo, the patient appeared well and reported mild improvement in her symptoms.

DISCUSSION

Video capsule endoscopy (VCE) has revolutionized noninvasive evaluation of small intestinal mucosa since its approval for clinical use in the United States in 2001. Initially utilized for the assessment of patients with GI bleeding of obscure origin, its indications have expanded to include evaluation for small bowel tumors, polyposis syndromes, inflammatory bowel disease, and enteropathies, including celiac disease[1]. Contraindications for the study include motility disorders, obstruction, known stenotic area in the GI tract, pregnancy[2]. Today, capsule endoscopy is used in evaluation of some esophageal and colonic disorders as well.

The PillCam® capsule (Given Imaging, Yoqneam, Israel) is 11 mm in diameter and 26 mm in length. Although VCE is generally regarded as safe[3,4], capsule retention is recognized as a major complication of the procedure, potentially leading to bowel obstruction requiring its surgical removal. The 4th International Conference on Capsule Endoscopy (ICCE) in 2005 defined capsule retention as "having a capsule endoscope remain in the digestive tract for a minimum of 2 wk". It was further defined as a "capsule remaining in the bowel lumen unless directed medical, endoscopic, or surgical intervention was instituted"[5]. The ICCE consensus did not set a time limit for removing retained capsules. In fact, asymptomatic retention and a possibility of reversing the cause of obstruction by directed medical management of the underlying condition may permit cautious non-surgical observation in select cases. Alternatively, double balloon enteroscopy may be helpful in removing retained capsule. The exact incidence of retained capsules varies widely from 0%[6] to 13%[7], depending on the indication for the study. Patients with obscure GI bleeding are considered low risk, in contrast to patients with active Crohn's disease or suspected small bowel obstruction where higher incidence of capsule retention was noted[7,8]. Careful history taking and small bowel radiologic evaluation will lead to anticipation of a potential obstruction, and use of biodegradable patency capsule may effectively assist in its precise localization. In general, a capsule retention requiring surgical intervention occurs at a rate of 0.75%[9], ultimately leading to correct diagnosis of the underlying pathology.

Review of the medical literature to date shows that the duration of asymptomatic capsule retention varies greatly from few weeks to several years. Anatomically, the video capsule is most often retained in the ileum[5,10]. Until now, the longest reported case was 38 mo in a patient with small bowel Crohn's disease[8]. To the best of our knowledge, our report describes the first case of a retained capsule in the stomach for over three years. It is also the first case report of adult hypertrophic pyloric stenosis diagnosed with video capsule endoscopy or a CT scan. In addition, we postulate that the physiologic worsening of our patient's symptoms over last three years was due to the added degree of gastric flow obstruction secondary to intermittent proximal "plugging" of the pyloric channel with a retained video capsule.

Hypertrophic pyloric stenosis (HPS) is a rare cause of gastric outlet obstruction. Infantile HPS is suspected in a neonate with projectile non-bilious vomiting and weight loss, and occurs in 0.2%-0.5% of births. Classically, "ol-

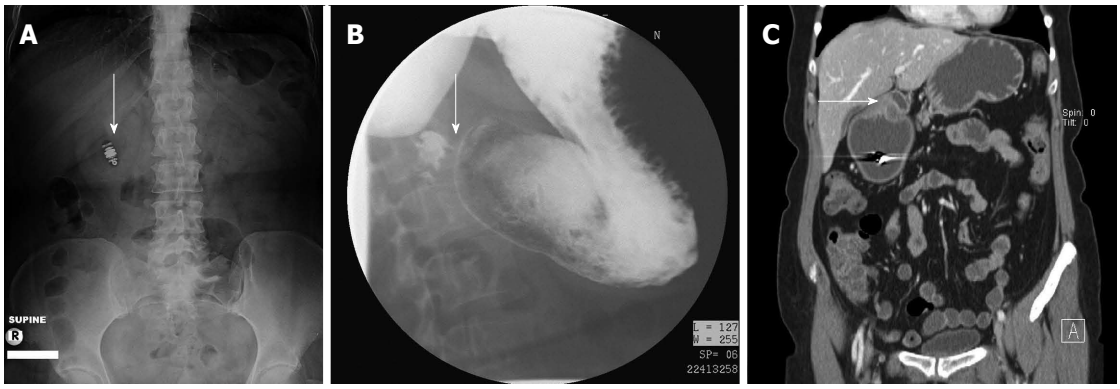

Figure 1 Radiologic imaging. A: Retained video capsule on abdominal roentogram (arrow); B: Barium upper gastrointestinal series (arrow) shows pronounced delay in gastric emptying with a narrowed slightly elongated pylorus and a distended gastric antrum; C: Computed enterography of the abdomen shows retained video capsule, distended antrum of the stomach, and abnormally dense, eccentric, and significantly narrowed pylorus (arrow).

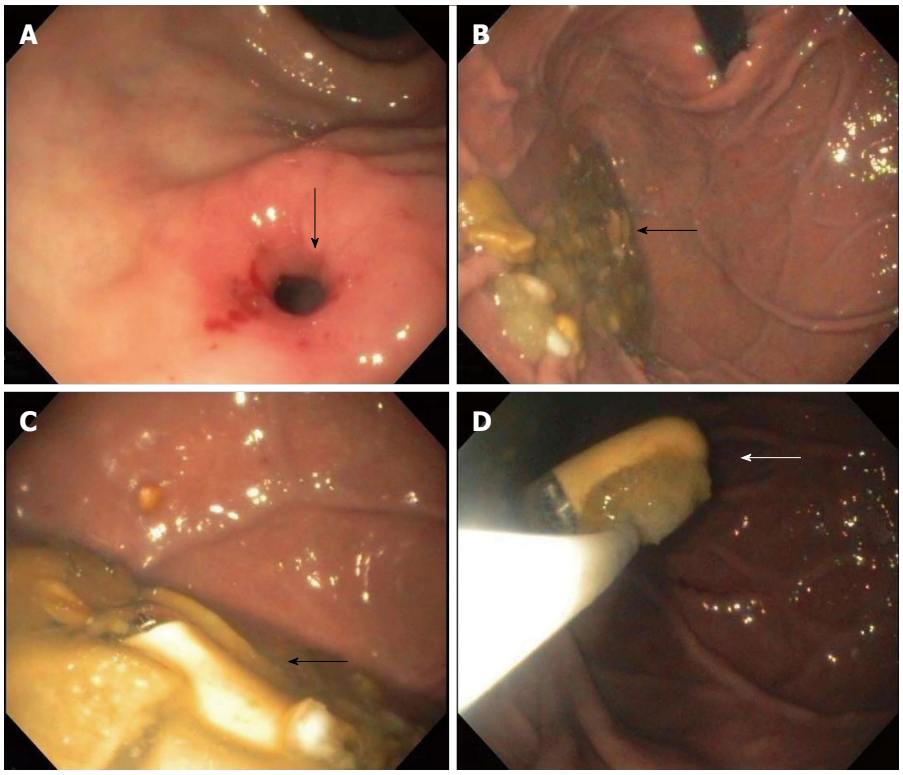

Figure 2 Endoscopic findings. A: Eccentric hypertrophic pyloric stenosis (arrow); B: Retroflexed view of the gastric fundus shows freely floating 3 years old video capsule in pool of retained semi-digested food particles (arrow); C: Close up view of retained capsule (arrow); D: Endoscopic retrieval with Roth Net (arrow).

ive sign" of a prominent pyloric muscle is palpated on physical examination and transabdominal ultrasound is diagnostic. Surgical intervention provides excellent prognosis. In contrast, adult HPS is a rare disorder that is further characterized as primary (idiopathic) or secondary (in association with peptic ulcer disease, malignancy, or hypertrophic gastropathy). To date, only over 200 cases of primary adult HPS have been described in the medical literature[11], although its prevalence is likely under-reported. Males are more likely to be affected, and although affected patients range from 14 to 85 years of age, it is mostly diagnosed in fourth and fifth decades of life[12]. It has been traditionally accepted that primary idiopathic adult HPS is likely a delayed presentation of a pediatric subclinical HPS. Histologically, there is marked hypertrophy and hyperplasia of the circular pyloric muscle. Grossly, pyloric channel greater than 1 cm in length and over 8 mm of muscular wall thickness is considered hypertrophic[13]. Expected diameter of normal pyloric orifice ranges from 1.2 to 1.5 cm[12]. Endoscopic findings may include "cervix sign" - a fixed narrowed pylorus with smooth borders that may preclude normal duodenal intubation with a standard gastroscope. Pyloric channel may also be eccentric in relation to the antrum with slight tenting towards lesser curvature. Upper GI series may demonstrate "Kirklin's sign" - a mushroom-like

deformity at the base of the duodenal bulb[11]. Clinically, patient may present with early satiety, abdominal fullness, nausea, epigastric discomfort and eructation. Vomiting of undigested foods may provide symptomatic improvement. Due to outlet obstruction, a gastric scintography may show delayed emptying, leading to a common misdiagnosis of gastroparesis and a delay in effective treatment. Management of symptomatic adult idiopathic HPS includes endoscopic balloon dilatation[14], surgical pyloroplasty or Billroth I or Ⅱ resection[11]. Potential benefit of Botulinum toxin injection in the pyloric sphincter may be of clinical interest, although its use in HPS has never been reported.

In conclusion, we report a first case in the medical literature of idiopathic adult HPS diagnosed by unusual finding of a retained video capsule endoscope in the stomach of the patient for over 3 years. This case raises a reasonable speculation that the use of a standard patency video capsule may, in certain cases, be applied to establish a diagnosis of occult HPS, differentiating it from an idiopathic gastroparesis, in which normal pyloric opening will eventually permit passage of the capsule into the small bowel. Permanent gastric retention of an 11 mm capsule may be detected by an abdominal roentogram and the device may be easily retrieved with an endoscope. It should be also noted that, specific to primary adult HPS, as large free floating capsule follows normal food bolus propagation it may effectively block an already compromised pyloric channel proximally, thus causing transient gastric outlet obstruction and appreciable worsening of patient's chronic symptoms. GI community should be aware of unique findings of capsule endoscopy in patients with HPS and use them to our advantage in arriving to correct diagnosis. In addition, advances in radiologic imaging have brought an important tool in CT enterography that, with better spatial resolution, may replace upper GI series as a test of choice in diagnosing primary HPS. Finally, this case raises clinical awareness of adult HPS, an uncommon medical condition that may mimic idiopathic gastroparesis, and importantly points out the need to vigilantly review antecedent workup in a complex patient with chronic GI symptoms to arrive to correct diagnosis.

REFERENCES

1 **Karagiannis S**, Faiss S, Mavrogiannis C. Capsule retention: a feared complication of wireless capsule endoscopy. *Scand J Gastroenterol* 2009; **44**: 1158-1165 [PMID: 19606392 DOI: 10.1080/00365520903132039]

2 **Kelley SR**, Lohr JM. Retained wireless video enteroscopy capsule: a case report and review of the literature. *J Surg Educ* 2009; **66**: 296-300 [PMID: 20005505 DOI: 10.1016/j.jsurg.2009.10.002]

3 **Scapa E**, Jacob H, Lewkowicz S, Migdal M, Gat D, Gluckhovski A, Gutmann N, Fireman Z. Initial experience of wireless-capsule endoscopy for evaluating occult gastrointestinal bleeding and suspected small bowel pathology. *Am J Gastroenterol* 2002; **97**: 2776-2779 [PMID: 12425547 DOI: 10.1111/j.1572-0241.2002.07021.x]

4 **Lewis BS**, Swain P. Capsule endoscopy in the evaluation of patients with suspected small intestinal bleeding: Results of a pilot study. *Gastrointest Endosc* 2002; **56**: 349-353 [PMID: 12196771 DOI: 10.1016/S0016-5107(02)70037-0]

5 **Cave D**, Legnani P, de Franchis R, Lewis BS. ICCE consensus for capsule retention. *Endoscopy* 2005; **37**: 1065-1067 [PMID: 16189792 DOI: 10.1055/s-2005-870264]

6 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterol Hepatol* 2005; **3**: 133-141 [PMID: 15704047 DOI: 10.1016/S1542-3565(04)00619-6]

7 **Cheifetz AS**, Lewis BS. Capsule endoscopy retention: is it a complication? *J Clin Gastroenterol* 2006; **40**: 688-691 [PMID: 16940879 DOI: 10.1097/00004836-200609000-00005]

8 **Cheifetz AS**, Kornbluth AA, Legnani P, Schmelkin I, Brown A, Lichtiger S, Lewis BS. The risk of retention of the capsule endoscope in patients with known or suspected Crohn's disease. *Am J Gastroenterol* 2006; **101**: 2218-2222 [PMID: 16848804 DOI: 10.1111/j.1572-0241.2006.00761.x]

9 **Barkin JS**, Friedman S. Wireless Capsule Endoscopy requiring surgical intervention: the world's experience. *Am J Gastroenterol* 2002; **97**: S298 [DOI: 10.1016/S0002-9270(02)05390-X]

10 **Cheon JH**, Kim YS, Lee IS, Chang DK, Ryu JK, Lee KJ, Moon JS, Park CH, Kim JO, Shim KN, Choi CH, Cheung DY, Jang BI, Seo GS, Chun HJ, Choi MG. Can we predict spontaneous capsule passage after retention? A nationwide study to evaluate the incidence and clinical outcomes of capsule retention. *Endoscopy* 2007; **39**: 1046-1052 [PMID: 18072054 DOI: 10.1055/s-2007-966978]

11 **Hellan M**, Lee T, Lerner T. Diagnosis and therapy of primary hypertrophic pyloric stenosis in adults: case report and review of literature. *J Gastrointest Surg* 2006; **10**: 265-269 [PMID: 16455460 DOI: 10.1016/j.gassur.2005.06.003]

12 **Knight CD**. Hypertrophic pyloric stenosis in the adult. *Ann Surg* 1961; **153**: 899-910 [PMID: 13757107 DOI: 10.1097/00000658-196106000-00010]

13 **Quigley RL**, Pruitt SK, Pappas TN, Akwari O. Primary hypertrophic pyloric stenosis in the adult. *Arch Surg* 1990; **125**: 1219-1221 [PMID: 2400314 DOI: 10.1001/archsurg.1990.01410210145025]

14 **Yusuf TE**, Brugge WR. Endoscopic therapy of benign pyloric stenosis and gastric outlet obstruction. *Curr Opin Gastroenterol* 2006; **22**: 570-573 [PMID: 16891891 DOI: 10.1097/01.mog.0000239874.13867.41]

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CASE REPORT

An ironic case of liver infections: Yersinia enterocolitis in the setting of thalassemia

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Abstract

A 49 years old Vietnamese male with a history of thalassemia, presented with gastrointestinal symptoms and signs of hemolysis. He was diagnosed with yersinia enterocolitis. Yersinia is a gram-negative rod that most frequently occurs in children especially during the winter months. In the current case, the bone marrow biopsy showed hemophagocytosis along with positive cultures for Yersinia. The microorganism likely triggered hemophagocytosis. This syndrome, also known as, hemophagocytic lymphohistiocytosis, is defined by fever for more than 7 d, cytopenia of two or more cell lines, hemophagocytosis, hepatitis, serum ferritin greater than 500, jaundice, lymphadenopathy, and hepatosplenomegaly. This disorder can be either familial or secondary to a strong immunologic activation. Both have an overwhelming activation of T-cells and macrophages.

Key words: Yersinia; Enterocolitis; Bone marrow; Liver biopsy; Thalassemia; Hemophagocytic lymphohistiocytosis

Core tip: In the current case, the bone marrow biopsy showed hemophagocytosis along with positive cultures for Yersinia. The microorganism likely triggered hemophagocytosis. This syndrome, also known as, hemophagocytic lymphohistiocytosis, is defined by fever for more than 7 d, cytopenia of two or more cell lines, hemophagocytosis, hepatitis, serum ferritin greater than 500, jaundice, lymphadenopathy, and hepatosplenomegaly. This disorder can be either familial or secondary to a strong immunologic activation. Both have an overwhelming activation of T-cells and macrophages.

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INTRODUCTION

Yersinia is a gram-negative rod that most frequently occurs in children especially during the winter months. Transmission is largely food and waterborne. Pigs are frequently colonized with strains that cause human illness. Incubation typically lasts 2-6 d followed by a diarrheal period that can last up to three weeks. Symptoms include nausea, vomiting, and abdominal pain. Most strains of Yersinia grow poorly in typical agar solutions because the bacteria lack a mechanism for the efficient uptake of iron. Individuals who have iron overload due to either primary or secondary hemochromatosis are at increased risk of infection, and are also at higher risk to develop severe infections. Complications of severe infection can include diffuse ulcerating ileitis and colitis, intussusception, perforation, toxic megacolon, cholangitis, mesenteric vein

thrombosis, and hemophagocytic lymphohistiocytosis. Post-infectious complications include erythema nodosum and reactive arthritis. Treatment, reserved only for severe systemic infections, should consist of a 3rd generation cephalosporin and gentamicin for 3 wk. Genetic studies on this patient showed a loss of three alpha globin genes indicating the presence of Hb H disease. This lack of alpha globin causes a relative increase in the number of beta globin chains which can aggregate to form unstable tetramers. The tetramers have abnormal oxygen dissociation curves reflected in poor delivery of oxygen to the periphery, as well as precipitation of the hemoglobin tetramers as Heinz bodies. These precipitants can induce phagocytosis of red blood cells and a chronic hemolytic anemia which in turn leads to an increase in serum hepcidin levels with resultant elevated iron transport across the gut mucosa. Over time, this leads to a systemic iron overload which can also be exacerbated iatrogenically by blood transfusions.

CASE REPORT

A 49-year-old Vietnamese male, with a history of malaria 27 years ago was well until 5 d prior to admission when he developed dark urine associated with fevers, chills, and night sweats. This was followed by non-bloody diarrhea, and right upper quadrant abdominal pain as well as nausea and non-bloody vomiting. He denied any IV drug abuse, sick contacts, or travel history. He drank alcohol socially, but not to excess. On physical exam, he had a temperature of 104.3 ℃, Blood pressure of 102/59 mmHg, heart rate of 100 beats/min, and saturation of 92% on room air. Generally, he was pale, diaphoretic, and sclerae were icteric. Abdominal examination revealed some right upper quadrant tenderness, but no rebound or guarding, and no hepatosplenomegaly. He had no rashes or stigmata of chronic liver disease. His laboratory studies showed a hemoglobin of 6.1 (13.8-18.0) g/dL with an MCV of 58 (80-100) fL, a white cell count of 10.6 (4.8-10.5) $10^3/\mu L$, and a platelet count of 75 (150-400) $10^3/\mu L$. Aspartate aminotransferase and alanine aminotransferase were 168 and 160 (5-40 and 7-56) U/L, respectively with a total bilirubin of 3.3 (0.3-1.9) mg/dL, and a direct bilirubin of 1.1 (0-0.3) mg/dL. Haptoglobin was < 15 (41-165). A peripheral smear demonstrated marked anisopoikilocytosis with schistocytes and target cells. Iron saturation was initially normal, 29%, with a ferritin of 6148 (12-300) mg/dL. Subsequent testing revealed persistently high iron saturation, 80%, and ferritin levels > 1000 mg/dL. Glucose and electrolytes were normal. Computerized tomography (CT) of the abdomen showed a normal biliary tree, proximal ascending colon mural thickening with surrounding adenopathy and pericolonic stranding as well as bilateral pleural effusions (Figure 1). Stool, blood, and bone marrow cultures were all positive for Yersinia enterocolitica. The patient was positive for HBsAg with a viral load of 270000 IU. Genetic testing revealed mutations of three alpha globin genes making a diagnosis of alpha thalassemia (Hb H). A liver biopsy showed 3+ iron in hepatocytes

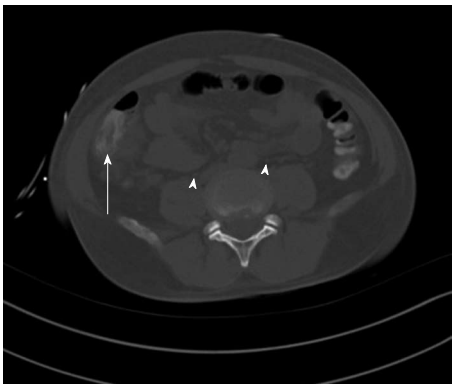

Figure 1 A computer tomography of the abdomen without contrast performed on the day of admission. There was moderate mural thickening of the proximal ascending colon (arrow) with surrounding adenopathy and mild pericolonic stranding. Also visible are mesenteric, pericolonic and retroperitoneal lymph nodes (arrowheads) with the largest measuring 1.6 cm in short axis in the right pericolonic region.

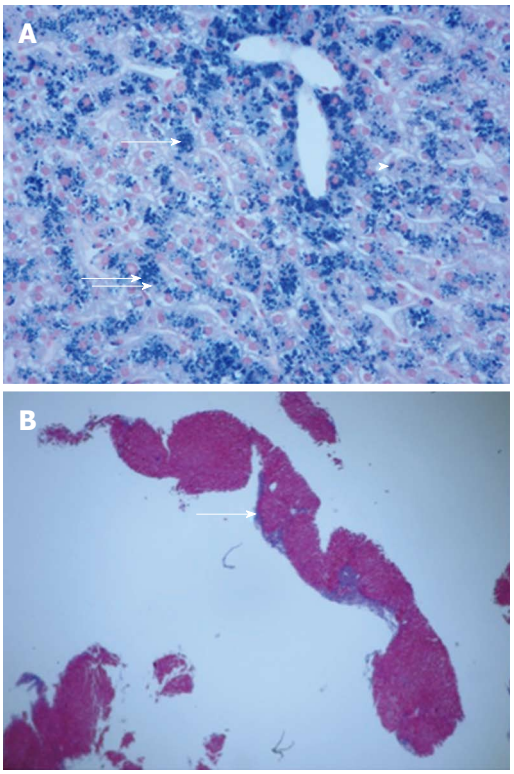

Figure 2 Liver biopsy. A: There is marked, 3+, accumulation of iron primarily in the hepatocytes (arrows), but also in Kupffer cells (arrow head), and bile duct epithelium in association with moderate lobular hepatitis (Prussian Blue stain for iron, × 400); B: There is increased fibrosis with focal portal-to-portal and occasional central-portal septum formation (arrow) indicating progression towards early cirrhosis (Masson Trichrome stain, × 40).

with a portal to central gradient (Figure 2A), and chronic inflammation with early septum formation (Figure 2B). Bone marrow biopsy revealed iron overload, and a granuloma (Figure 3A), and hemophagocytic lymphohistiocytosis (Figure 3B)[1]. The patient was started on piperacillin/tazobactam/gentamicin and transfused to a hemoglobin level of 10 g/dL with rapid clinical improvement. He was discharged on a 3-wk course of oral ciprofloxacin, and a

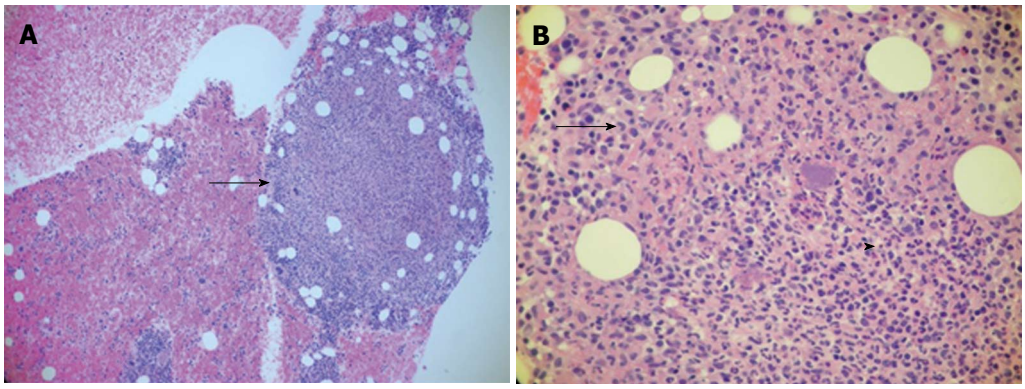

Figure 3 Bone marrow biopsy. A: A necrotizing granuloma (arrow) with trilineage maturation and markedly increased iron storage [hematoxylin and eosin (HE) stain, ×100]; B: An area of necrosis (arrowhead) with erythrophagocytosis typical, but not diagnostic of Yersinia infection (HE stain, × 400).

follow up CT of the abdomen showed resolution of the bowel thickening and disappearance of the fat stranding. In addition, he was treated with oral deferasirox (Exjade) and entecavir. His ferritin level decreased to 842 by 12 wk. His liver enzyme levels returned to normal, and his HBV viral load became undetectable.

DISCUSSION

In the current case, the bone marrow biopsy showed hemophagocytosis along with positive cultures for Yersinia. The microorganism likely triggered hemophagocytosis[2]. This syndrome, also known as, hemophagocytic lymphohistiocytosis, is defined by: fever for more than 7 d, cytopenia of two or more cell lines, hemophagocytosis, hepatitis, serum ferritin greater than 500, jaundice, lymphadenopathy, and hepatosplenomegaly. This disorder can be either familial or secondary to a strong immunologic activation. Both have an overwhelming activation of T-cells and macrophages.

In this patient, the chronic anemia due to thalassemia, or anemia in combination with the hepatitis B caused a secondary hemochromatosis[3]. This increased the risk of Yersinia infection, and likely was responsible for the severity of the systemic infection[4].

This patient will need iron chelation therapy and close monitoring for development of hepatocellular carcinoma because of the heightened risk with his coexisting HBV infection.

REFERENCES

1 **Au WY**, Lam WW, Chu WW, Tam S, Wong WK, Lau J, Yeung YM, Liu HS, Liang R. Organ-specific hemosiderosis and functional correlation in Chinese patients with thalassemia intermedia and hemoglobin H disease. *Ann Hematol* 2009; **88**: 947-950 [PMID: 19165482 DOI: 10.1007/s00277-009-0701-2]

2 **Pallister C**, Rotstein OD. Yersinia enterocolitica as a cause of intra-abdominal abscess: the role of iron. *Can J Surg* 2001; **44**: 135-136 [PMID: 11308237]

3 **Sebastiani G**, Tempesta D, Alberti A. Hepatic iron overload is common in chronic hepatitis B and is more severe in patients coinfected with hepatitis D virus. *J Viral Hepat* 2012; **19**: e170-e176 [PMID: 22239515 DOI: 10.1111/j.1365-2893.2011.01508.x]

4 **Xu L**, Chen EQ, Lei J, Liu L, Zhou TY, Gao Z, Tang H. Precore/basal-core promoter and reverse transcriptase mutations in chronic HBV infected-patients. *Hepatogastroenterology* 2012; **59**: 212-215 [PMID: 22251541]

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CASE REPORT

A case of plasmablastic lymphoma of the liver without human immunodeficiency virus infection

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Abstract

Plasmablastic lymphoma (PBL) is a very rare B-cell lymphoproliferative disorder was with an aggressive clinical behavior that recently characterized by the World Health Organization. Although PBL is most commonly observed in the oral cavity of human immunodeficiency virus (HIV)-positive patients, it can also be observed at extra-oral sites in HIV-negative patients. Epstein-Barr virus (EBV) may be closely related the pathogenesis of PBL. PBL shows different clinicopathological characteristics between HIV-positive and -negative patients. Here, we report a case of PBL of the liver in a 79-year-old HIV-negative male. The patient died approximately 1.5 mo after examination and autopsy showed that the main lesion was a very large liver mass. Histopathological examination of the excised lesion showed large-cell lymphoma with plasmacytic differentiation diffusely infiltrating the liver and involving the surrounding organs. The neoplastic cells were diffusely positive for CD30, EBV, Bob-1, and CD38. The autopsy findings suggested a diagnosis of PBL. To our knowledge, the present case appears to be the first report of PBL with initial presentation of the liver in a patient without HIV infection.

Key words: Plasmablastic lymphoma; Human immunodeficiency virus-negative; Normal liver; Pathogenesis; Immunohistochemistry

Core tip: Plasmablastic lymphoma (PBL) is a rare B-cell lymphoma. Although PBL is observed in the oral cavity of human immunodeficiency virus (HIV)-positive patients, it can also be observed at extra-oral sites in HIV-negative patients. We present a case of PBL of the liver in a 79-year-old HIV-negative male. Histopathological examination showed large-cell lymphoma with plasmacytic differentiation diffusely infiltrating the liver and involving the surrounding organs. The neoplastic cells were diffusely positive for CD30, Epstein-Barr virus, Bob-1, and CD38. The present case appears to be the first report of PBL with initial presentation in the liver in a patient without HIV infection.

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INTRODUCTION

Plasmablastic lymphoma (PBL) is a distinct, aggressive B-cell neoplasm that shows a diffuse proliferation of large neoplastic cells resembling B-immunoblasts with

an immunophenotype of plasma cells. PBL was initially described in 1997 as a rare subtype of diffuse large B-cell lymphoma (DLBCL) and has an aggressive clinical behavior arising in the oral cavity of human immunodeficiency virus (HIV)-infected individuals[1]. There have been several reports of PBL in HIV-negative patients, mainly at extra-oral sites including the stomach, small intestine and colon[2-6] but not in the liver. Interestingly, most cases of PBL occur without HIV infection in Japan and Korea, where the prevalence of HIV infection is low in comparison to Western countries[7,8]. In the present study, we describe the first case of liver PBL in a 79-year-old HIV-negative Japanese patient.

CASE REPORT

A 79-year-old Japanese man was admitted to our hospital with the chief complaint of abdominal pain and icterus. Upon physical examination, jaundice was found to be prominent, which gradually worsened without relief of symptoms. Abdominal examination revealed the liver was palpable about 10 cm below the costal margin. The transaminase, gamma glutamyl transpeptidase, alkaline phosphatase, and bilirubin levels all notably exceeded normal values. An abdominal ultrasound showed dilatation of the intrahepatic bile ducts and a heterogeneous large mass that was 15 cm in diameter located in the right hepatic lobe. Radiographs and computed tomography (CT) of the chest, oral, and peri-oral sites showed no abnormalities, and no peripheral lymphadenopathy was observed. Serological tests to detect specific antibodies against hepatitis B and C virus were negative. The carcinoembryonic antigen and alpha-fetoprotein levels were normal, but soluble interleukin-2 receptor was high (5760 U/mL), and the patient tested negative for an anti-HIV antibody. The examination of the serum levels of Bence-Jones protein and rheumatoid factor was also negative. The patient's medical history included a gastrectomy for a gastric ulcer 20 years earlier and a hepatectomy for a liver abscess 5 years earlier. Plain (Figure 1A) and enhanced (Figure 1B) abdominal CT revealed a liver tumor and hepatomegaly with the same lesion present on ultrasonography (US).

Following examination, a US percutaneous-guided fine needle liver biopsy was performed. Histopathological examination of the biopsy smears showed a dense infiltrate composed of diffuse and proliferative large immunoblast cells with a typical morphological appearance of high-grade non-Hodgkin's lymphoma. However, we were unable to make a diagnosis due to poor immunostaining and a low number of specimens. During the detailed examination, CT and US revealed tumor progression with jaundice, despite continued percutaneous transhepatic biliary drainage.

Considering the high tumor burden and poor prognosis, the patient chose not to receive chemotherapy. He received palliative care and passed away due to multiple organ failure 1.5 mo after his initial clinical presentation. An autopsy was performed immediately after his death. Gross findings included a white, soft solid tumor of approximately diameter of 10 cm in the liver that involved the diaphragm and parietal peritoneum (Figure 2A). Histologically, large tumor cells with abundant basophilic cytoplasm were diffusely proliferative, and large nuclei were sporadically or centrally located and contained one or two conspicuous nucleoli in the central portion (Figure 2B). Specifically, the tumor cells had plasmablast- or immunoblast-like morphology, and there were almost no mature plasma cells present (Figure 2C). Additionally, binucleated or multinucleated tumor cells were scattered throughout the specimen, tumor cell proliferation in lymphatic vessels was conspicuous, and tumor cell invasion was found in some blood vessels.

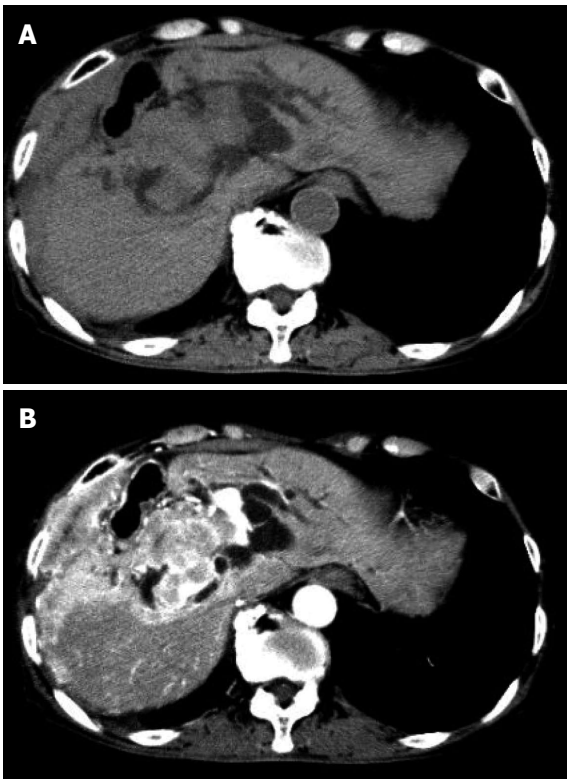

Figure 1 Computed tomography showing dilatation of the intrahepatic bile ducts and a heterogeneous large mass 15 cm in diameter located in the right hepatic lobe. A: Plain computed tomography (CT); B: Contrast-enhanced CT.

Immunohistochemical examination revealed that the tumor cells were negative for B-cell markers CD20 (Figure 3A) and CD3 (Figure 3B) but positive for CD30 (Figure 3C), Epstein-Barr virus (EBV) (Figure 3D), and Bob-1 (Figure 3E). Although over 90% of the tumor cells were positive for Ki-67, they were negative for epithelial cell markers such as AEI/AE3, S100 protein, CD43, CD56, PAX-5, and human herpes virus (HHV)-8. Almost all tumor cells were highly positive for EBV virus-encoded RNA *in situ* hybridization (EBER-ISH). Based on these results, a diagnosis of PBL was made.

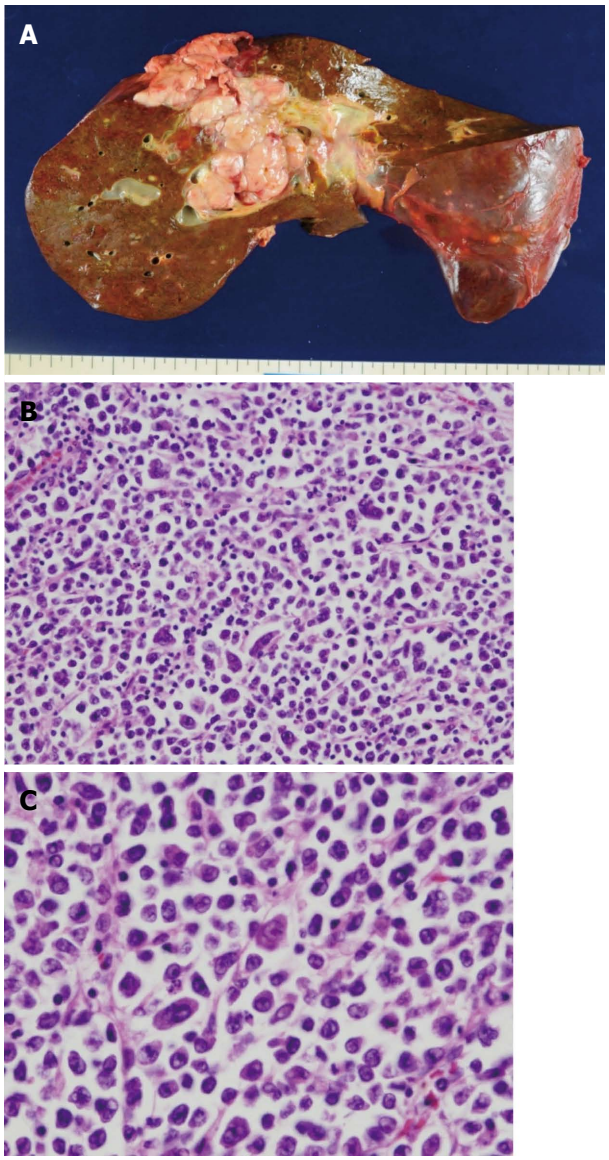

Figure 2 Gross appearance of the primary tumor lesion at autopsy and histological appearance. A: A large tumor (diameter of 10 cm) is present in the liver. The cut section shows that the tumor is white and solid, with marked necrosis; B: Diffuse infiltration in the liver by monotonous large atypical lymphoid cells (HE staining; original magnification × 200); C: These atypical cells have an abundant basophilic cytoplasm, eccentrically located pleomorphic nuclei, and single, centrally located prominent nucleoli (HE; original magnification × 400).

DISCUSSION

DLBCL is currently considered to be a heterogeneous group of rare tumors primarily occurring in the presence of HIV infection[9,10]. DLBCL with plasmablastic features has recently been categorized into the following subtypes: PBL of the oral cavity, PBL with plasmacytic differentiation, classic primary effusion lymphoma (PEL), extracavitary/solid PEL or HHV8-associated DLBCL, and anaplastic lymphoma kinase-positive DLBCL[10,11].

PBL is a rare lymphoma accounting for approximately 2.6% of AIDS-related neoplasms[12] and typically occurs in the oral cavity of HIV-infected patients. Recently, however, PBL has been reported in patients without HIV infection, and several cases have been reported in extra-oral locations, such as skin, subcutaneous tissue, stomach, anal mucosa, lung, and lymph node regions[3-6,13,14]. PBL has also been observed in immunocompromised individuals such as organ transplant patients and the elderly. PBL with HIV infection primarily affects men at a young age, while the clinical characteristics of PBL without HIV infection are typically old age with a slight male predominance[15]. This difference reflects the epidemiology of HIV infection. In addition, over one-third of all cases with PBL were first noted at extra-oral locations[16]. Interestingly, although the gastrointestinal tract has been observed to be the most common extraoral site (10.6%), the liver is a rare extra-oral location in PBL patients[16]. Only one case of liver PBL patient with HIV infection has been reported[17].

PBL is a distinct, aggressive B-cell neoplasm that shows a diffuse proliferation of large neoplastic cells resembling B-immunoblasts with an immunophenotype of plasma cells. PBL cells predominantly exhibit the morphological features of plasmablasts/immunoblasts and are immunohistologically negative or slightly positive for B-cell markers such as CD20 and CD79a but positive for plasmacyte markers such as CD38 and CD138. PBL cells are highly reactive to the cell proliferation marker Ki67, and 2 in 3 patients carry an integrated EBV genome, while HHV8 is negative. EBER-ISH is highly positive in PBL cells; in particular, EBER-ISH has a positive predictive value of close to 100% in HIV-positive PBL patients with presentation in the oral cavity[18,19].

Differential diagnosis is needed for poorly differentiated carcinomas and malignant melanomas, in addition to malignant lymphomas, such as anaplastic (plasmablastic) plasmacytoma (AP), Burkitt's lymphoma, and DLBCL-NOS[20]. Although a differential diagnosis of AP is especially difficult because of tumor cell morphology and immunohistochemical results that are similar to other subtypes, AP cases are usually preceded by a plasmacytoma such as multiple myeloma.

Because the positive value of EBER is significantly high in PBL patients, it has been suggested to use this value as a diagnostic tool[18]. However, some plasmacytomas with no signs of immune system abnormalities exhibit plasmablast-like morphology with a particularly high EBER-positive rate[21]. To differentiate the two diseases, it is important to use clinical findings (such as the presence of multiple myeloma and M protein) in addition to histopathological and immunohistological findings. The overall prognosis of PBL is poor [12,15]; however, the prognosis of PBL in HIV-positive patients has improved with the enhanced management of HIV symptoms, but it has worsened in HIV-negative patients, which include a large elderly population[7,15]. MYC/IgH translocation is observed in 70% of EBER-positive PBL patients, and, interestingly, these patients have a notably worse PBL prognosis, presenting with an endemic Burkitt lymphoma-like phenotype and symptoms[22,23].

The management of PBL is based mainly on early

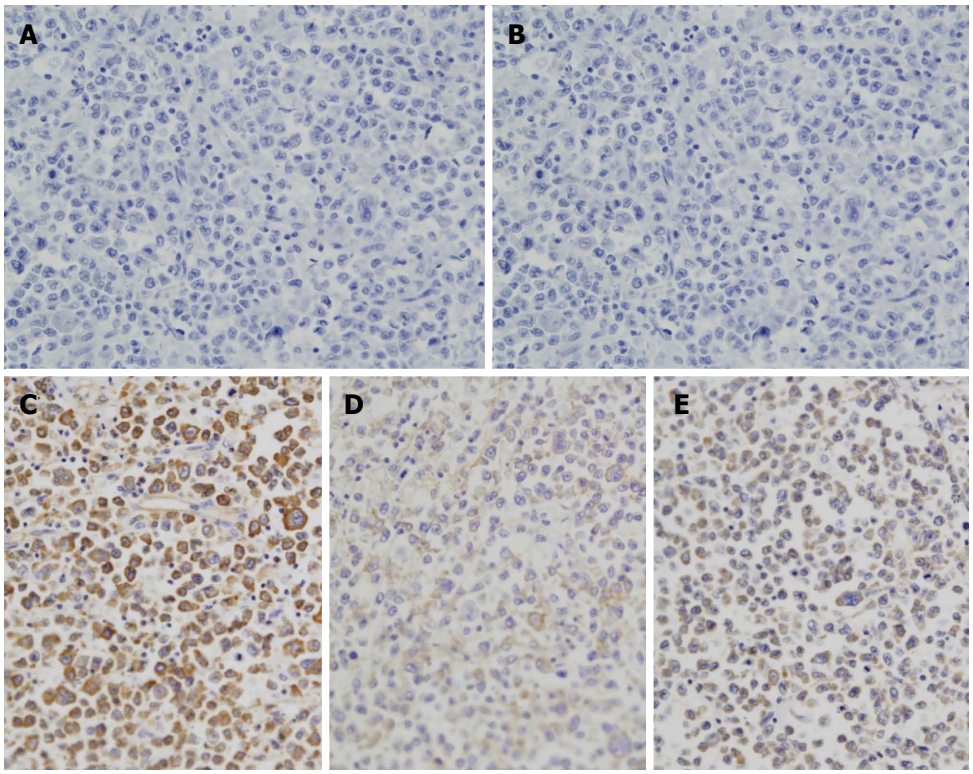

Figure 3 Immunohistochemical staining showing tumor cells with a negative expression of CD20 (A) and CD3 (B), positive expression of CD30 (C), Epstein-Barr virus (D), and Bob-1 (E).

aggressive chemotherapy. Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and CHOP-like regimens are commonly used with a good overall response rate but have a high relapse rate and poor overall survival[24].

In our case, no primary carcinoma was detected in any organs at autopsy. In cases of malignant melanoma, tumor cells are immunohistochemically positive for melanosomes and S-100 protein, and Burkitt's lymphomas expresses LCA, CD20, and CD79a. A differential diagnosis between PBL and plasmacytoma is often difficult without histological examination. Because PBL is composed almost entirely of blast cells, plasmacytoma typically consists of mature plasma cells. Therefore, the morphology and immunophenotype of both tumor cells, as well as clinical features, are essential for an accurate diagnosis of PBL.

The condition of the patient rapidly deteriorated because we failed to provide proper treatment due to the unsuccessful initial histopathological examination of the liver biopsy. If biopsy specimens show similar pathological features to those presented in this study, a differential diagnosis of PBL and an immunohistochemical analysis of CD138 and EBER are urgently needed. Although there is one reported case of PBL originating in the liver[17], to our knowledge, this is the first case report of PBL that occurred in the liver without HIV infection. Because extra-oral PBL can occur in HIV-negative patients and has a poor prognosis, it should be included as a differential diagnosis in cases of suspected hepatic lymphoma.

REFERENCES

1 **Delecluse HJ**, Anagnostopoulos I, Dallenbach F, Hummel M, Marafioti T, Schneider U, Huhn D, Schmidt-Westhausen A, Reichart PA, Gross U, Stein H. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood* 1997; **89**: 1413-1420 [PMID: 9028965]

2 **Masgala A**, Christopoulos C, Giannakou N, Boukis H, Papadaki T, Anevlavis E. Plasmablastic lymphoma of visceral cranium, cervix and thorax in an HIV-negative woman. *Ann Hematol* 2007; **86**: 615-618 [PMID: 17361427 DOI: 10.1007/s00277-007-0280-z]

3 **Chabay P**, De Matteo E, Lorenzetti M, Gutierrez M, Narbaitz M, Aversa L, Preciado MV. Vulvar plasmablastic lymphoma in a HIV-positive child: a novel extraoral localisation. *J Clin Pathol* 2009; **62**: 644-646 [PMID: 19561233 DOI: 10.1136/jcp.2009.064758]

4 **Hatanaka K**, Nakamura N, Kishimoto K, Sugino K, Uekusa T. Plasmablastic lymphoma of the cecum: report of a case with cytologic findings. *Diagn Cytopathol* 2011; **39**: 297-300 [PMID: 20607680 DOI: 10.1002/dc.21420]

5 **Liu JJ**, Zhang L, Ayala E, Field T, Ochoa-Bayona JL, Perez L, Bello CM, Chervenick PA, Bruno S, Cultrera JL, Baz RC, Kharfan-Dabaja MA, Raychaudhuri J, Sotomayor EM, Sokol L. Human immunodeficiency virus (HIV)-negative plasmablastic lymphoma: a single institutional experience and literature review. *Leuk Res* 2011; **35**: 1571-1577 [PMID: 21752466 DOI: 10.1016/j.leukres.2011.06.023]

6 **Tavora F**, Gonzalez-Cuyar LF, Sun CC, Burke A, Zhao XF. Extra-oral plasmablastic lymphoma: report of a case and review of literature. *Hum Pathol* 2006; **37**: 1233-1236 [PMID: 16938530 DOI: 10.1016/j.humpath.2006.04.009]

7 **Teruya-Feldstein J**, Chiao E, Filippa DA, Lin O, Comenzo R, Coleman M, Portlock C, Noy A. CD20-negative large-cell

lymphoma with plasmablastic features: a clinically heterogenous spectrum in both HIV-positive and -negative patients. *Ann Oncol* 2004; **15**: 1673-1679 [PMID: 15520070 DOI: 10.1093/annonc/mdh399]
8 **Yotsumoto M**, Ichikawa N, Ueno M, Higuchi Y, Asano N, Kobayashi H. CD20-negative CD138-positive leukemic large cell lymphoma with plasmablastic differentiation with an IgH/MYC translocation in an HIV-positive patient. *Intern Med* 2009; **48**: 559-562 [PMID: 19336959]
9 **De Paepe P**, De Wolf-Peeters C. Diffuse large B-cell lymphoma: a heterogeneous group of non-Hodgkin lymphomas comprising several distinct clinicopathological entities. *Leukemia* 2007; **21**: 37-43 [PMID: 17039226 DOI: 10.1038/sj.leu.2404449]
10 **Carbone A**, Gloghini A. AIDS-related lymphomas: from pathogenesis to pathology. *Br J Haematol* 2005; **130**: 662-670 [PMID: 16115121 DOI: 10.1111/j.1365-2141.2005.05613.x]
11 **Teruya-Feldstein J**. Diffuse large B-cell lymphomas with plasmablastic differentiation. *Curr Oncol Rep* 2005; **7**: 357-363 [PMID: 16091196]
12 **Carbone A**, Gloghini A. Plasmablastic lymphoma: one or more entities? *Am J Hematol* 2008; **83**: 763-764 [PMID: 18756546 DOI: 10.1002/ajh.21259]
13 **Takahashi Y**, Saiga I, Fukushima J, Seki N, Sugimoto N, Hori A, Eguchi K, Fukusato T. Plasmablastic lymphoma of the retroperitoneum in an HIV-negative patient. *Pathol Int* 2009; **59**: 868-873 [PMID: 20021612 DOI: 10.1111/j.1440-1827.2009.02457.x]
14 **Chuah KL**, Ng SB, Poon L, Yap WM. Plasmablastic lymphoma affecting the lung and bone marrow with CD10 expression and t(8; 14)(q24; q32) translocation. *Int J Surg Pathol* 2009; **17**: 163-166 [PMID: 18508845 DOI: 10.1177/1066896908316785]
15 **Castillo JJ**, Winer ES, Stachurski D, Perez K, Jabbour M, Milani C, Colvin GA, Butera JN. HIV-negative plasmablastic lymphoma: not in the mouth. *Clin Lymphoma Myeloma Leuk* 2011; **11**: 185-189 [PMID: 21575922 DOI: 10.1016/j.clml.2011.03.008]
16 **Riedel DJ**, Gonzalez-Cuyar LF, Zhao XF, Redfield RR, Gilliam BL. Plasmablastic lymphoma of the oral cavity: a rapidly progressive lymphoma associated with HIV infection. *Lancet Infect Dis* 2008; **8**: 261-267 [PMID: 18353267 DOI: 10.1016/s1473-3099(08)70067-7]
17 **Metta H**, Corti M, Maranzana A, Schtirbu R, Narbaitz M, De Dios Soler M. Unusual case of plasmablastic non-Hodgkin's lymphoma located in the liver. First case reported in an AIDS patient. *Ann Hepatol* 2009; **8**: 242-245 [PMID: 19841505]
18 **Vega F**, Chang CC, Medeiros LJ, Udden MM, Cho-Vega JH, Lau CC, Finch CJ, Vilchez RA, McGregor D, Jorgensen JL. Plasmablastic lymphomas and plasmablastic plasma cell myelomas have nearly identical immunophenotypic profiles. *Mod Pathol* 2005; **18**: 806-815 [PMID: 15578069 DOI: 10.1038/modpathol.3800355]
19 **Dong HY**, Scadden DT, de Leval L, Tang Z, Isaacson PG, Harris NL. Plasmablastic lymphoma in HIV-positive patients: an aggressive Epstein-Barr virus-associated extramedullary plasmacytic neoplasm. *Am J Surg Pathol* 2005; **29**: 1633-1641 [PMID: 16327436]
20 **Colomo L**, Loong F, Rives S, Pittaluga S, Martínez A, López-Guillermo A, Ojanguren J, Romagosa V, Jaffe ES, Campo E. Diffuse large B-cell lymphomas with plasmablastic differentiation represent a heterogeneous group of disease entities. *Am J Surg Pathol* 2004; **28**: 736-747 [PMID: 15166665]
21 **Chang ST**, Liao YL, Lu CL, Chuang SS, Li CY. Plasmablastic cytomorphologic features in plasma cell neoplasms in immunocompetent patients are significantly associated with EBV. *Am J Clin Pathol* 2007; **128**: 339-344 [PMID: 17638671 DOI: 10.1309/27h8xjh31f3gunat]
22 **Valera A**, Balagué O, Colomo L, Martínez A, Delabie J, Taddesse-Heath L, Jaffe ES, Campo E. IG/MYC rearrangements are the main cytogenetic alteration in plasmablastic lymphomas. *Am J Surg Pathol* 2010; **34**: 1686-1694 [PMID: 20962620 DOI: 10.1097/PAS.0b013e3181f3e29f]
23 **Bogusz AM**, Seegmiller AC, Garcia R, Shang P, Ashfaq R, Chen W. Plasmablastic lymphomas with MYC/IgH rearrangement: report of three cases and review of the literature. *Am J Clin Pathol* 2009; **132**: 597-605 [PMID: 19762538 DOI: 10.1309/ajcpfur1bk0uodts]
24 **Castillo JJ**, Winer ES, Stachurski D, Perez K, Jabbour M, Milani C, Colvin G, Butera JN. Prognostic factors in chemotherapy-treated patients with HIV-associated Plasmablastic lymphoma. *Oncologist* 2010; **15**: 293-299 [PMID: 20167839 DOI: 10.1634/theoncologist.2009-0304]

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CASE REPORT

Composite diffuse large B-cell lymphoma and classical Hodgkin's lymphoma of the stomach: Case report and literature review

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Abstract

The combination of classical Hodgkin's lymphoma (cHL) and non-Hodgkin lymphoma coexisting in the same patient is not common, especially in one extranodal location. Here we present a rare case of composite diffuse large B-cell lymphoma (DLBCL) and cHL occurring simultaneously in the stomach of a 53-year-old female who presented with upper abdominal discomfort and gas pain. Surgery was performed and the disease was diagnosed pathologically as composite lymphoma of DLBCL and cHL using hematoxylin-eosin and immunohistochemical staining. Epstein-Barr virus (EBV) infection was not detected by *in situ* hybridization for EBV-encoded RNA or immunohistochemistry for EBV latent membrane protein-1. Polymerase chain reaction analysis from the two distinct components of the tumor demonstrated clonal immunoglobulin κ light chain gene rearrangements. The patient died approximately 11 mo after diagnosis in spite of receiving eight courses of the CHOP and two courses of the rituximab-CHOP (RCHOP) chemotherapy regimen. This case report showed that the two distinct components, DLBCL and cHL, appeared to originate from the same clonal progenitor cell, and that EBV infection was not essential for transformation during the course of tumorigenesis.

Key words: Composite lymphoma; Diffuse large B-cell lymphoma; Hodgkin's lymphoma; Stomach

Core tip: Classical Hodgkin's lymphoma (cHL) commonly manifests in lymph nodes whereas primary extranodal cHL in the gastrointestinal tract is very rare, and only single cases of primary gastric cHL have been reported in the literature. The combination of cHL and non-Hodgkin lymphoma (NHL) coexisting in the same patient is not common, especially in one extranodal location. The combination of cHL and NHL coexisting in the stomach is extremely rare. Here we present a case of composite diffuse large B-cell lymphoma and mixed cellularity cHL involving the stomach, and present a review of the literature.

Wang HW, Yang W, Wang L, Lu YL, Lu JY. Composite diffuse large B-cell lymphoma and classical Hodgkin's lymphoma of the stomach: Case report and literature review. *World J Gastroenterol* 2013; 19(37): 6304-6309 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6304.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6304

INTRODUCTION

Composite lymphoma (CL), which is defined as the coexistence of two or more morphologically and phenotypically distinct lymphoma types in a single anatomic organ or tissue, is unusual[1]. Almost all the primary stomach

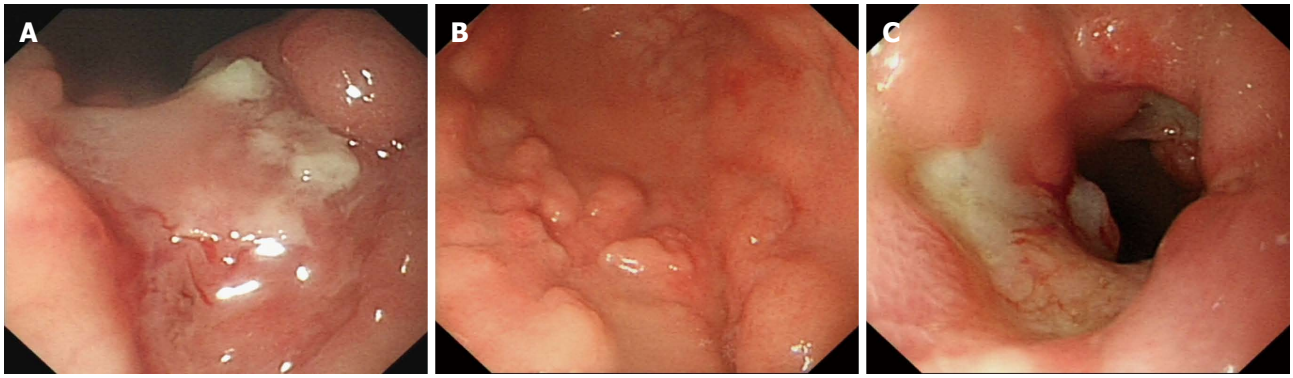

Figure 1 Gastroscopy showing an irregular ulcer covered with white exudates (A) and multiple mucosal nodularities in the gastric corpus (B) and a circular ulcer in the gastric pyloric canal (C).

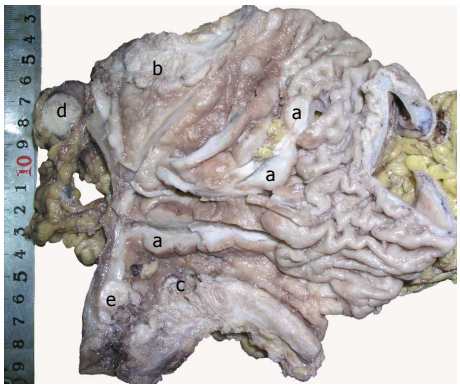

Figure 2 Macroscopic findings of the lesions. Multiple mucosal nodularities (a) and an ulcer (b) in the gastric corpus, a circular ulcer in the gastric pyloric canal (c), perigastric (d) and parapyloric (e) swollen lymph nodes.

lymphomas are non-Hodgkin's lymphoma (NHL), the majority of which are of B-cell origin, and mucosa-associated lymphoid tissue lymphoma and diffuse large B-cell lymphoma (DLBCL) account for over 90%[2]. Classical Hodgkin's lymphoma (cHL) commonly manifests in lymph nodes whereas primary extranodal cHL in the gastrointestinal tract is very rare, estimated at 0.025% of all cHL, and only single cases of primary gastric cHL have been reported in the literature[3]. The combination of cHL and NHL coexisting in the stomach is extremely rare. Here we present a rare case of composite DLBCL and mixed cellularity cHL involving the stomach, and present a review of the literature.

CASE REPORT

A 53-year-old female presented with upper abdominal discomfort and flatulent pain for over an 8-mo period and her condition became gradually worse. She also described a substantial weight loss and anorexia over the preceding 6 mo. Computed tomography (CT) scans of the abdomen showed thickening of the wall in the gastric pylorus and gastric corpus, and uneven enhancement could be seen after intravenous administration of contrast agent. Gastroscopy revealed an irregular ulcer covered with white exudates (Figure 1A) and multiple mucosal nodularities in the gastric corpus (Figure 1B). Another circular ulcer covered with white exudates and effusion was simultaneously found in the gastric pyloric canal (Figure 1C). Biopsy specimens were obtained from the two ulcers. A histologic diagnosis of small round cell malignant tumor, indicating lymphoma, was made. Routine blood examination showed hemoglobin 115 g/L, white blood cell count 5.32 × 10^9/L, neutrophils 52.3%, lymphocytes 19.6%, monocytes 12.1%. Serological testing demonstrated negativity for hepatitis B virus and human immunodeficiency virus infections. The lactate dehydrogenase level (185 U/L) was in the normal range. Abdominal ultrasonography and computed tomography scans of the chest did not show any other abnormalities. No superficial lymphadenopathy was noted.

The patient underwent distal stomach resection because of aggravated symptoms of obstruction. Grossly, the greater and lesser curvatures of the resected stomach measured 17.0 and 7.5 cm respectively. The gastric wall was diffusely thickened and multiple different sizes of mucosal nodularities ranging from 1 cm to 2.5 cm in diameter (Figure 2, a) and a well circumscribed ulcer measuring 4 cm × 3.5 cm × 0.5 cm (Figure 2, b) were identified in the gastric corpus. The gastric pyloric canal presented with increased thickness and stenosis, and a circular ulcer measuring 3 cm × 3 cm × 0.8 cm was also found (Figure 2, c). The cut surface of the neoplastic ulcers and mucosal nodularities were grey and soft. Perigastric (Figure 2, d) and parapyloric (Figure 2, e) swollen lymph nodes were identified. Selected tumor tissues were fixed in formalin and embedded in paraffin and cut into sections then stained with hematoxylin and eosin for routine histology. Additional sections of paraffin-embedded tissue were used for immunohistochemical staining and *in situ* hybridization analysis. Genomic DNA was isolated from $CD30^+$ Hodgkin and Reed-Sternberg (RS) cells and $CD20^+$ DLBCL cells by micromanipulation, and polymerase chain reaction (PCR) procedures were performed for analysis of immunoglobulin heavy and κ light chain rearrangements.

Microscopically, there were two morphologically and

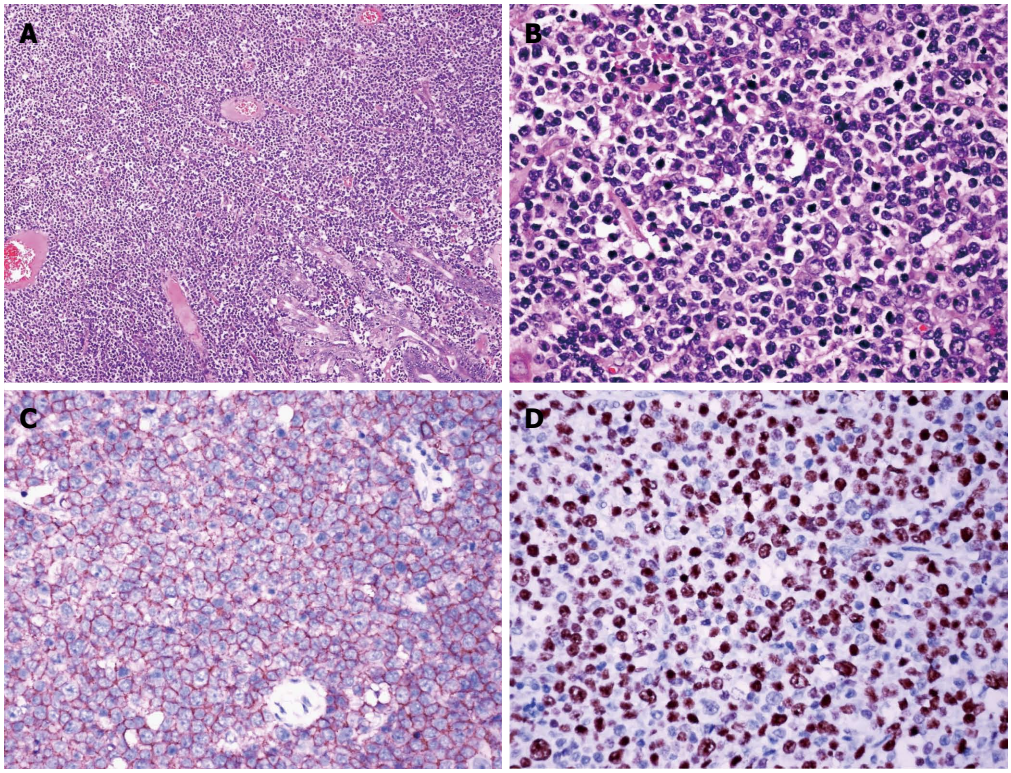

Figure 3 Diffuse large B-cell lymphoma of the stomach. A: Large lymphoid cells diffusely infiltration the gastric corpus wall (HE, × 100); B: Nucleoli and frequent mitotic figures (HE, × 400); C: Neoplastic cells diffusely positive for CD20 (immunoperoxidase stain, × 400); D: Nuclear proliferation rate as assessed by Ki-67 staining was approximately 80% (immunoperoxidase stain, × 400). HE: Hematoxylin and eosin.

immunophenotypically distinct components in different locations of the stomach. The ulcer and multiple mucosal nodularities in the gastric corpus exhibited a homogeneously uniform population of large lymphoid cells infiltration all layers of the gastric wall (Figure 3A). The nuclei were round or multilobated, with finely dispersed chromatin and evident nucleoli. Frequent mitotic figures were noted (Figure 3B). The neoplastic cells showed uniform expression of CD45, CD20 (Figure 3C), CD79a, Pax-5, MUM1, and absence of CD3, Bcl-6 and CD10. The nuclear proliferation rate as assessed by Ki-67 staining was approximately 80% (Figure 3D). Additional immunohistochemistry displayed tumor cells negative for cytokeratin, CD30, CD15 and other T-cell antigens. The ulcer in the gastric pylorus showed typical mixed lymphocyte, eosinophil granulocyte and neutrophil granulocyte infiltration with fibrosis (Figure 4A), and contained numerous large atypical lymphoid cells, including Hodgkin and RS cells (Figure 4B). The Hodgkin and RS cells were positive for CD30 (Figure 4C), CD15 (Figure 4D), MUM1 and Oct-2, and weakly positive for Pax-5, but negative for CD45, CD20, CD79a, CD3, CD10 and BOB.1. Interestingly, the perigastric and parapyloric swollen lymph nodes were infiltrated by tumor cells of DLBCL and cHL, respectively. Neither cell population showed markers of Epstein-Barr virus (EBV) infection by *in situ* hybridization for EBV-encoded RNA or immunohistochemistry for EBV latent membrane protein-1. On the basis of these morphologic and immunohistochemical characteristics, the pathological diagnosis of composite DLBCL and mixed cellularity cHL was made. PCR analysis from the two distinct components of the tumor demonstrated clonal immunoglobulin κ light chain gene rearrangements (Figure 5).

After surgery, the patient was treated with eight courses of a standard CHOP (cyclophospamide, doxorubicin, vincristine, and prednisone) chemotherapy regimen, after which she showed an excellent response with normal brain, thoracic and abdominal CT scans. Unfortunately, repeat CT scans and ultrasonography revealed tumor recurrence with abdominal tumor load 7 mo after chemotherapy. Then the patient received a further two cycles of rituximab-CHOP (RCHOP) chemotherapy. Unfortunately, she died of multiple organ failure due to lymphoma recurrence on the 11th postoperative month. An autopsy was not performed.

DISCUSSION

The concept of CL was first put forward by Custer[4] to explain the occurrence of more than one histological type of lymphoma in the same patient. In the study of more than 1000 cases for the International Working Formulation for NHL, the incidence of CL varied between 1% and 4.7%[5]. cHL and NHL are morphologically and clinically distinct neoplasms. The combination of cHL and NHL coexisting in the same tissue is rare and much more uncommon than other combinations[3]. According

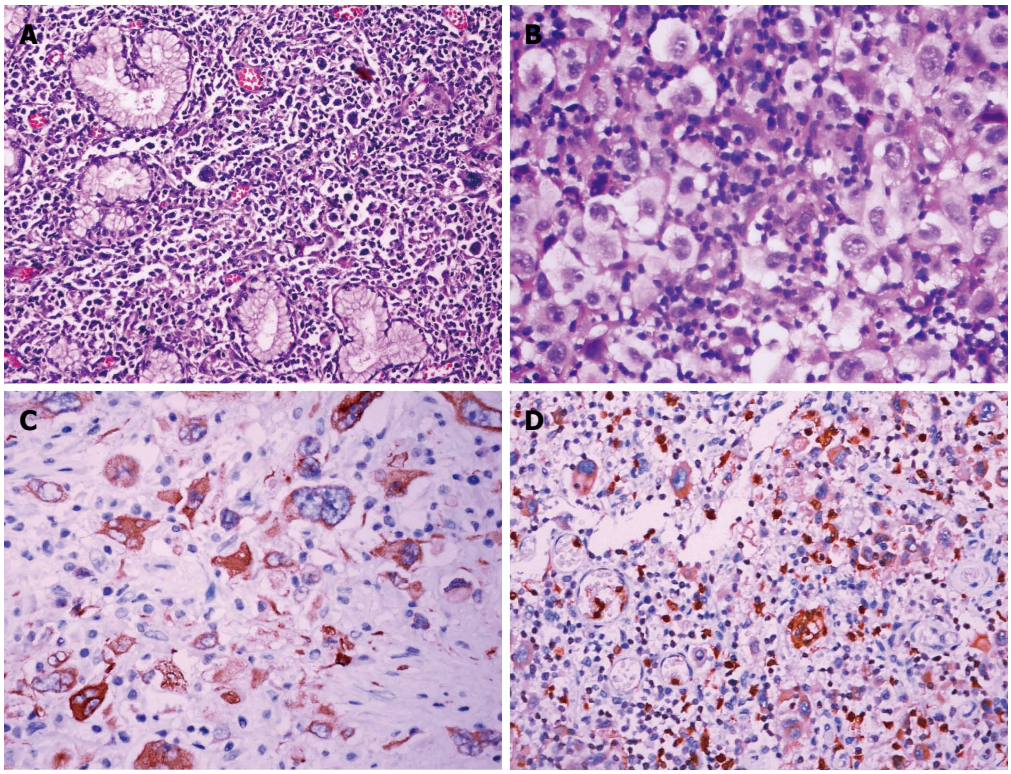

Figure 4 Classical Hodgkin's lymphoma of the stomach. A: Mixed lymphocyte, eosinophil granulocyte and neutrophil granulocyte infiltrateing the gastric pyloric canal wall (HE, × 200); B: Hodgkin and Reed-Sternberg (RS) cells are present (HE, × 400); C, D: Hodgkin and RS cells positive for CD30 and CD15, respectively (immunoperoxidase stain, × 400). HE: Hematoxylin and eosin.

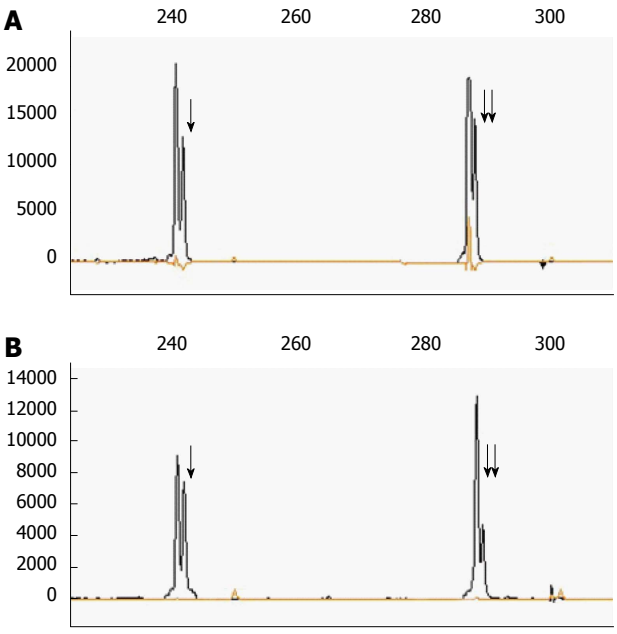

Figure 5 Polymerase chain reaction analysis from the two distinct components of the tumor demonstrated clonal immunoglobulin κ light chain gene rearrangements. The asterisks indicate two peaks representing the rearranged polymerase chain reaction products from position 241 bp (arrow) and 281 bp (double arrows) regions of immunoglobulin κ light chain gene, respectively. A: DNA from the dissected diffuse large B-cell lymphoma component. B: DNA from the dissected classical Hodgkin lymphoma component.

to our literature review, a total of 10 cases, including the present case, reported a combination of DLBCL and cHL within the same site simultaneously[6-13]. The clinical data of all previously published cases of composite DLBCL and cHL are listed in Table 1. In these cases, there were five cases of combination of DLBCL and cHL in the lymph nodes, three cases in the stomach, one case in the small intestine and one case in the anterior mediastinum, indicating that the gastrointestinal tract is the most common extranodal site involved in this kind of composite lymphoma. Prochorec-Sobieszek *et al*[9] firstly reported localized gastric DLBCL and cHL as secondary neoplasms in two patients with chronic lymphocytic leukemia. To the best of our knowledge, this is the first case report of composite DLBCL and cHL coexisting in the stomach with no history of lymphoma or leukemia.

The pathogenesis of CL is inconclusive. Viral infections, genetic susceptibility, genetic mutations, and immune suppression are CL pathogenic factors. However, no single definite mechanism has been proposed to explain the pathogenesis of different types of CL as the etiology is variable, complex and differs according to the types of lymphomas involved[14-16]. In general, cHL is associated with EBV; on the other hand, NHL is infrequently associated with EBV. When cHL and NHL are present in the same anatomic site, there is a higher correlation with the presence of EBV in both lymphoma

Table 1 Composite diffuse large B-cell lymphoma and classical Hodgkin's lymphoma: A review of the literature

Ref.	Gender/age (yr)	Organ	EBV infection	Treatment	Follow up time	Status
Paulli *et al*[6]	Male/37	Supra-clavicular lymph nodes	NA	8 cycles of pro-MACE-CytaBOM chemotherapy	23 wk	ANED
Bellan *et al*[7]	Female/29	Cervical lymph nodes	NA	MACOP-B chemotherapy for 8 wk and autologous stem cells transplant	30 mo	ANED
Rosenquist *et al*[8]	Female/74	Inguinal lymph nodes	+	6 cycles of CHOP chemotherapy	12 wk	ANED
Prochorec-Sobieszek *et al*[9]	Male/67	Stomach	+	6 courses of CHOP chemotherapy	22 wk	ANED
	Male/76	Stomach	+	6 courses of cyclophosphamide and cladribine chemotherapy	14 wk	DOD
Huang *et al*[10]	Male/56	Small intestine	+	Lesion resected	6 d	DOD
Miyagaki *et al*[11]	Male/75	Axillary lymph nodes	+	6 courses of RCHOP chemotherapy	3 yr	DOD
Yu *et al*[12]	Female/37	Anterior mediastinum	–	6 courses of CHOP chemotherapy and 23 times radiotherapy	33 wk	ANED
Bautista-Quach *et al*[13]	Female/6	Multiple lymph nodes	+	Combined chemotherapy (program unknown)	17 wk	DOD
Wang *et al* (present case)	Female/53	Stomach	–	Lesion resected and 8 courses of CHOP and 2 courses of RCHOP chemotherapy	45 wk	DOD

NA: Not available; +: Positive; -: Negative; CHOP: Cyclophosphamide, doxorubicin, vincristine, and prednisone; RCHOP: Rituximab-CHOP; ANED: Alive with no evidence of disease; DOD: Died of disease.

cells than when two lymphomas occur at different times and/or at different sites. If the two components demonstrate positivity for EBV, it would be suggested that a commonly infected progenitor cell might be responsible for both lymphomas[17,18]. From assessment of the data in Table 1, composite DLBCL and cHL often showed EBV positivity, suggesting an origin from a commonly EBV-infected progenitor cell; however, two components from two cases including our patient were all negative for EBV, indicating EBV infection did not seem to be the primary event in this tumorigenesis.

Lymphoma, generally, is defined as monoclonal proliferation of lymphocytes (T cell, B cell or natural killer cell). Coexistence of DLBCL and cHL in the same anatomic location has been reported occasionally; studies using molecular techniques have proved that they may be clonally related (*i.e.*, derived from the same lymphoid progenitors) or not related (*i.e.*, different lymphoid progenitors)[7-10]. Controversy about this issue may reflect the lack of a full understanding of the pathogenesis of these lymphomas or the heterogeneity of Hodgkin lymphoma and NHL. In the present case, identical immunoglobulin κ light chain gene rearrangements were seen in the two distinct components, indicating that both components, despite their distinctly different morphologic features and immunophenotype, were indeed derived from the same clone. Thus, DLBCL and cHL coexisting in the stomach in our case can be considered a true CL with two distinctive presentations.

The pathological differential diagnosis of composite DLBCL and cHL in the present case mainly included cHL transformation to DLBCL, anaplastic variant of DLBCL, T cell/histiocyte-rich large B-cell lymphoma (THRLBCL), anaplastic large cell lymphoma (ALCL) and grey zone lymphoma. The characteristic morphology and immunophenotype of the tumor cells in conjunction with clinical features aid in the differential diagnosis. cHL transformation to DLBCL may be differentiated from the current case as the two distinct lymphoma occurred simultaneously within different sites of the stomach without a histological mixing zone. An anaplastic variant of DLBCL is characterized by large tumor cells with bizarre pleomorphic nuclei that may resemble Hodgkin and/or RS cells, and it may be differentiated according to its consistent immunological staining for B-cell markers, such as CD20 and CD79. THRLBCL is comprised of scattered, single, large B cells embedded in a background of T cells and a variable number of histiocytes. These large B cells may mimic Hodgkin and RS cells in cHL, but they express pan B-cell markers with no expression of CD15 and CD30. The "Hodgkin-like pattern" accounts for 3% of ALCL cases, which is characterized by morphological feathers mimicking nodular sclerosis cHL. CD15 expression is rarely observed and when present only a small proportion of the neoplastic cells are stained; however, Hodgkin and RS cells in cHL are always weakly positive for Pax-5, which is different from ALCL. The most presentation of grey zone lymphoma is a large anterior mediastinal mass with rare involvement of non-lymphoid organs. Some areas may more closely resemble DLBCL and others appear more like cHL. In cases that morphologically resemble cHL, uniform strong expression of CD20 and other B-cell markers and absence of CD15 would favor the diagnosis of grey zone lymphoma. Other histological differential diagnoses, including leukocythemia, poorly differentiated carcinoma, sarcoma, reactive lymphoid proliferation and collision tumor should be cautiously considered[19].

To conclude, we report a rare case of composite DLBCL and cHL involving the stomach and describe the histologic and immunophenotypic findings. The contribution of immunohistochemistry plays an important role in differential diagnosis. Using molecular techniques, we further proved that the two different components ap-

peared to originate from the same clonal progenitor cell, and that EBV infection was not essential for transformation during the course of tumorigenesis.

REFERENCES

1 **Mokhtar NM**. Review article composite lymphoma. *J Egypt Natl Canc Inst* 2007; **19**: 171-175 [PMID: 19190689]
2 **Ghimire P**, Wu GY, Zhu L. Primary gastrointestinal lymphoma. *World J Gastroenterol* 2011; **17**: 697-707 [PMID: 21390139 DOI: 10.3748/wjg.v17.i6.697]
3 **Zettl A**, Rüdiger T, Marx A, Müller-Hermelink HK, Ott G. Composite marginal zone B-cell lymphoma and classical Hodgkin's lymphoma: a clinicopathological study of 12 cases. *Histopathology* 2005; **46**: 217-228 [PMID: 15693895 DOI: 10.1111/j.1365-2559.2005.02046.x]
4 **Custer RP**. Pitfalls in the diagnosis of lymphoma and leukemia from pathologist's point of view. Proceedings of Second National Conference. New York: American Cancer Society, 1954: 554-557
5 **Thirumala S**, Esposito M, Fuchs A. An unusual variant of composite lymphoma: a short case report and review of the literature. *Arch Pathol Lab Med* 2000; **124**: 1376-1378 [PMID: 10975943]
6 **Paulli M**, Rosso R, Kindl S, Boveri E, Sirchi M, De Medici A, Invernizzi R, Magrini U. Nodular sclerosing Hodgkin's disease and large cell lymphoma. Immunophenotypic characterization of a composite case. *Virchows Arch A Pathol Anat Histopathol* 1992; **421**: 271-275 [PMID: 1384224 DOI: 10.1007/BF01611185]
7 **Bellan C**, Lazzi S, Zazzi M, Lalinga AV, Palummo N, Galieni P, Marafioti T, Tonini T, Cinti C, Leoncini L, Pileri SA, Tosi P. Immunoglobulin gene rearrangement analysis in composite hodgkin disease and large B-cell lymphoma: evidence for receptor revision of immunoglobulin heavy chain variable region genes in Hodgkin-Reed-Sternberg cells? *Diagn Mol Pathol* 2002; **11**: 2-8 [PMID: 11854595 DOI: 10.1097/00019606-200203000-00002]
8 **Rosenquist R**, Menestrina F, Lestani M, Küppers R, Hansmann ML, Bräuninger A. Indications for peripheral light-chain revision and somatic hypermutation without a functional B-cell receptor in precursors of a composite diffuse large B-cell and Hodgkin's lymphoma. *Lab Invest* 2004; **84**: 253-262 [PMID: 14688797 DOI: 10.1038/labinvest.3700025]
9 **Prochorec-Sobieszek M**, Majewski M, Sikorska A, Kos K, Huszcza S, Maryniak RK. Localized gastric diffuse large B-cell lymphoma and Hodgkin's lymphoma as secondary neoplasms in two patients with chronic lymphocytic leukemia. *Leuk Lymphoma* 2006; **47**: 2244-2246 [PMID: 17071501 DOI: 10.1080/10428190600763546]
10 **Huang Q**, Wilczynski SP, Chang KL, Weiss LM. Composite recurrent hodgkin lymphoma and diffuse large B-cell lymphoma: one clone, two faces. *Am J Clin Pathol* 2006; **126**: 222-229 [PMID: 16891197 DOI: 10.1309/DDGLWRV3KR9164G7]
11 **Miyagaki T**, Sugaya M, Minatani Y, Fujita H, Hangaishi A, Kurokawa M, Takazawa Y, Tamaki K. Mycosis fungoides with recurrent Hodgkin's lymphoma and diffuse large B-cell lymphoma. *Acta Derm Venereol* 2009; **89**: 421-422 [PMID: 19688163 DOI: 10.2340/00015555-0663]
12 **Yu G**, Kong L, Qu G, Zhang Q, Wang W, Jiang L. Composite lymphoma in the anterior mediastinum: a case report and review of the literature. *Diagn Pathol* 2011; **6**: 60 [PMID: 21733146 DOI: 10.1186/1746-1596-6-60]
13 **Bautista-Quach MA**, Bedros A, Wang J. Subsequent development of diffuse large B-cell lymphomas and Hodgkin lymphoma associated with primary immune disorder in a 6-year-old female: a case report and review of the literature. *J Pediatr Hematol Oncol* 2011; **33**: e320-e325 [PMID: 21572347 DOI: 10.1097/MPH.0b013e318207a37a]
14 **Hirose Y**, Fukushima T, Masaki Y, Shimoyama K, Karasawa H, Ogawa N, Wano Y. Epstein-Barr virus-associated composite lymphoma composed of peripheral T-cell lymphoma and an anaplastic variant of a diffuse large B-cell type of non-Hodgkin's lymphoma and strongly expressing p53 protein. *Int J Hematol* 2004; **79**: 260-265 [PMID: 15168595 DOI: 10.1532/IJH97.03156]
15 **Carbone A**, Gloghini A. AIDS-related lymphomas: from pathogenesis to pathology. *Br J Haematol* 2005; **130**: 662-670 [PMID: 16115121 DOI: 10.1111/j.1365-2141.2005.05613.x]
16 **Falchi L**, Capello D, Palumbo B, Rauco A, Emili R, Cianciulli M, Pace R, Capparella V, Liberati F, Liberati AM. A case of nodular sclerosis Hodgkin's lymphoma repeatedly relapsing in the context of composite plasma cell-hyaline vascular Castleman's disease: successful response to rituximab and radiotherapy. *Eur J Haematol* 2007; **79**: 455-461 [PMID: 17908180 DOI: 10.1111/j.1600-0609.2007.00952.x]
17 **Guarner J**, del Rio C, Hendrix L, Unger ER. Composite Hodgkin's and non-Hodgkin's lymphoma in a patient with acquired immune deficiency syndrome. In-situ demonstration of Epstein-Barr virus. *Cancer* 1990; **66**: 796-800 [PMID: 2167145]
18 **Harada S**, Kalla H, Balasubramanian M, Brodsky I, Gladstone D, Hou JS. Classical Hodgkin lymphoma concurrently evolving in a patient with marginal zone B-cell lymphoma of the spleen. *Ann Diagn Pathol* 2008; **12**: 212-216 [PMID: 18486899 DOI: 10.1016/j.anndiagpath.2006.12.005]
19 **El Demellawy D**, Ross C, Sur M, Alowami S. Synchronously diagnosed lymph nodal collision tumor of malignant melanoma and chronic lymphocytic leukemia/small lymphocytic lymphoma: case report. *Diagn Pathol* 2007; **2**: 34 [PMID: 17760975 DOI: 10.1186/1746-1596-2-34]

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CASE REPORT

Giant biliary cystadenoma complicated with polycystic liver: A case report

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Author contributions: Yang ZZ collected the clinical data and wrote the paper; Li Y, Yan YH, Liu J and Xiao WD performed the operation; Li KF assisted in the reference search.

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Abstract

Biliary cystadenoma (BCA) is a rare hepatic neoplasm. Although considered a benign cystic tumor of the liver, BCA has a high risk of recurrence with incomplete excision and a potential risk for malignant degeneration. Correct diagnosis and complete tumor excision with negative margins are the mainstay of treatment. Unfortunately, due to the lack of presenting symptoms, and normal laboratory results in most patients, BCA is hard to distinguish from other cystic lesions of the liver such as biliary cystadenocarcinoma, hepatic cyst, hydatid cyst, Caroli disease, undifferentiated sarcoma, intraductal papillary mucinous tumor, and hepatocellular carcinoma. Ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) may be necessary. They demonstrate intrahepatic cystic lesions with features such as mural nodules, varying wall thickness, papillary projections, and internal septations. Nevertheless, surgery is still the only means of accurate diagnosis. Definitive diagnosis requires histological examination following formal resection. We describe a 57-year-old woman initially diagnosed with polycystic liver who was subsequently diagnosed with giant intrahepatic BCA in the left hepatic lobe. This indicates that both US physicians and hepatobiliary specialists should attach importance to hepatic cysts, and CT or MRI should be performed for further examination when a diagnosis of BCA is suspected.

Key words: Biliary cystadenoma; Diagnosis; Hepatic cysts; Ultrasound

Core tip: We present a case of a 57-year-old woman who was diagnosed with polycystic liver ten years ago. She had intermittent abdominal discomfort and pain in the past 2 years. Last month, she was admitted to our hospital, and underwent exploratory laparotomy with left hepatic lobectomy, right liver cyst fenestration, and cholecystectomy. She was then diagnosed with giant biliary cystadenoma complicated with polycystic liver.

Yang ZZ, Li Y, Liu J, Li KF, Yan YH, Xiao WD. Giant biliary cystadenoma complicated with polycystic liver: A case report. *World J Gastroenterol* 2013; 19(37): 6310-6314 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i37/6310.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i37.6310

INTRODUCTION

Biliary cystadenoma (BCA) is a rare hepatic neoplasm. Although considered a benign cystic tumor of the liver, BCA has a high risk of recurrence with incomplete excision and a potential risk for malignant degeneration. Correct diagnosis and complete tumor excision with negative margins are the mainstay of treatment. Unfortunately, due to the lack of presenting symptoms, and normal laboratory results in most patients, it is hard to distinguish BCA from other cystic lesions of the liver. Definitive diagnosis requires histological examination following for-

mal resection. We describe a 57-year-old woman initially diagnosed with polycystic liver who was subsequently diagnosed with a giant intrahepatic BCA in the left hepatic lobe. This indicates that importance should be attached to hepatic cysts in the ultrasound (US) examination, and computed tomography (CT) or magnetic resonance imaging (MRI) should be performed for further examination when a diagnosis of BCA is suspected.

CASE REPORT

A 57-year-old woman was admitted to our hospital with intermittent abdominal discomfort and pain for almost 2 years. Discomfort and pain were not related to meals, defecation or change in position, and could be tolerated. Initially, she underwent an US examination of the abdomen at a local hospital. This revealed multiple small cysts in the liver, which caused no particular concern. She had not experienced diarrhea, nausea, vomiting, fever or chills since the onset of symptoms. However, she noticed that her abdominal girth appeared to be slowly increasing in size 1 mo ago, and repeat US examination at an outside institution showed a left hepatic multiloculated cystic mass measuring 21.0 cm × 9.1 cm × 13.6 cm with internal septations and multiple small cysts in the left liver lobe.

She visited our hospital for further treatment. On physical examination, it was significant for abdominal tenderness and the abdomen was distended, with a large, soft, non-mobile mass in the left half of the abdomen. The patient had no history of intravenous drug use or tattoos or body piercing, no history of excessive alcohol use or obesity, and no history of working with toxic chemicals. She had no prior history of surgery, medical illness, and no known allergies. She was not using any medication. There was no significant family history of biliary or liver diseases.

CT imaging performed at our institution demonstrated a left hepatic multiloculated cystic mass measuring 15.0 cm × 9.1 cm, occupying the majority of the upper abdomen, and normal liver structure had disappeared. The internal septations were visible and enhanced after intravenous administration of contrast medium. The mass cranially displaced the liver. The gallbladder and pancreas were compressed. Simultaneously, multiple sizes of hypoattenuating shadows without enhancement were seen in the right liver lobe (Figure 1). Abdominal MRI was ordered and revealed a large cystic tumor measuring approximately 18.0 cm × 9.0 cm originating from the left liver lobe (Figure 2). On T1-weighted imaging (T1WI), low signal intensity was apparent within the cystic spaces. On corresponding T2-weighted imaging (T2WI), the tumor was characterized by a medium-high intensity signal clearly delineated from the surrounding liver tissue with internal septal structures separating the fluid-filled spaces.

Laboratory tests were within normal limits, and serology for hepatitis B virus infection was negative, and serum carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, CA-125 and α-fetoprotein (AFP) levels were normal. On the basis of these findings, the patient was diagnosed with a hepatobiliary cystadenoma and polycystic liver.

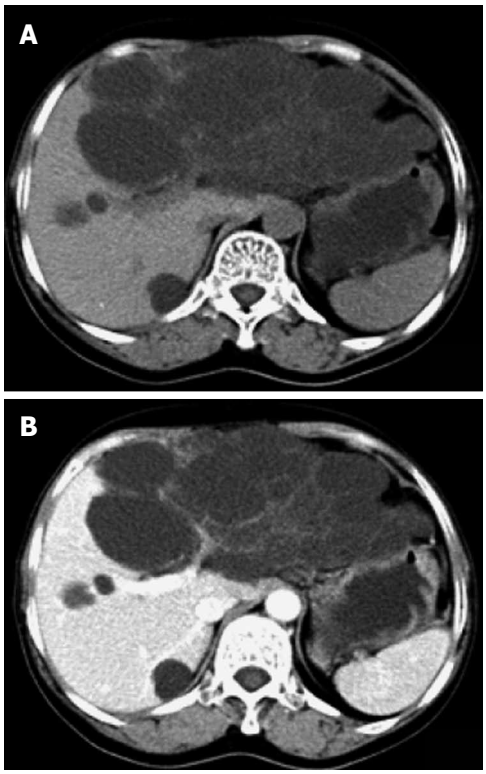

Figure 1 Transverse computed tomography scan showed a left hepatic multiloculated cystic mass measuring 15.0 cm × 9.1 cm (A) and contrast computed tomography showing enhanced septum of the tumor (B). Simultaneously, multiple sizes of hypoattenuating shadows without enhancement were seen in the right liver lobe.

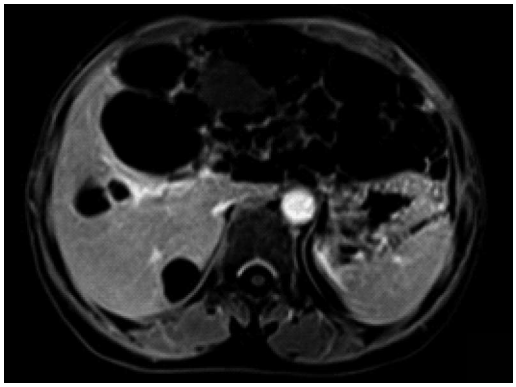

Figure 2 T1-weighted imaging revealed a large cystic tumor measuring approximately 18.0 cm × 9.0 cm originating from the left liver lobe.

The patient underwent an exploratory laparotomy with left hepatic lobectomy, right liver cyst fenestration, and cholecystectomy through a right subcostal incision. A large cystic mass presenting with grape-like blisters was located on the surface of the left lobe of the liver. It was well encapsulated and essentially without invasion into any other structures, and it was completely excised.

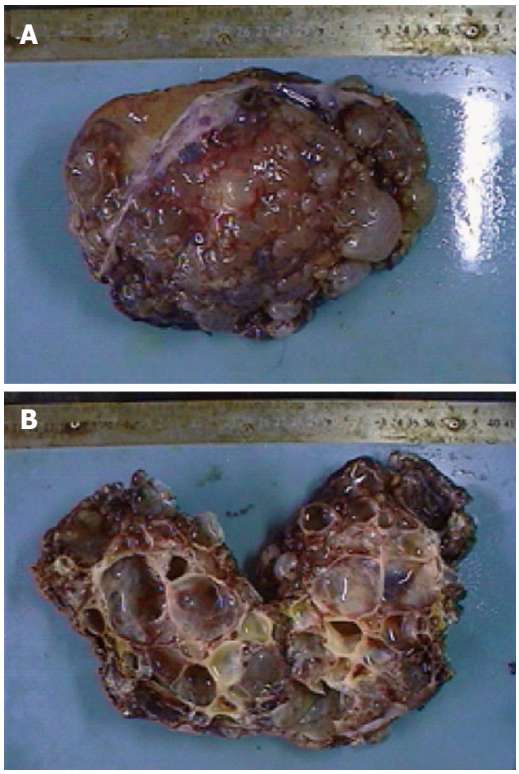

Figure 3 Resected left liver specimen showed a multilocular cystic lesion measuring 15 cm × 9 cm × 8 cm, covered with bullate nodules on the cut surface (A), and opened specimen filled with grayish yellow but clear fluid, the inner surface was smooth without any masses or excrescences (B).

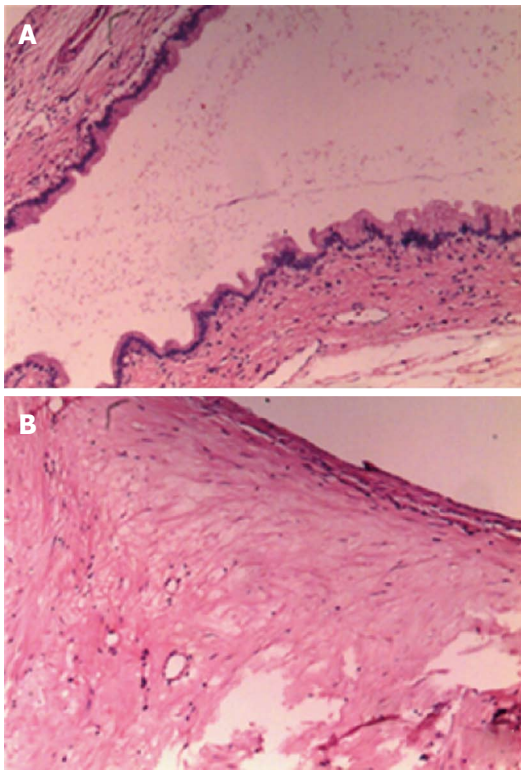

Figure 4 Microscopic evaluation showed a single layer of cuboidal to columnar epithelial cells (A) with underlying stroma with proliferating fibrous tissue and a small number of inflammatory cells (B) (hematoxylin and eosin stain, × 100).

On gross examination, the resected left liver specimen showed a multilocular cystic lesion measuring 15 cm × 9 cm × 8 cm, covered with bullate nodules on the cut surface (Figure 3A). The cyst contained grayish yellow but clear fluid with no connection to the bile duct, and the wall was smooth. No masses or excrescences were noted on the inner surface (Figure 3B). Microscopically, the cystic lesion was lined by a single layer of cuboidal to columnar epithelial cells (Figure 4A). The cell morphology was normal and not pleomorphic. A stroma with proliferating fibrous tissue and a small number of inflammatory cells was underlying the epithelium (Figure 4B). Typical ovarian-like stroma was absent. The histopathology was homogeneous and uniform throughout the lesion. The surgical margins were negative and a final diagnosis of hepatobiliary cystadenoma was established. It also showed chronic inflammation of the gallbladder of the excised specimen.

The patient was seen 1 mo after surgery in the clinic. She was able to eat normally and had no abdominal discomfort. She was monitored with abdominal US for recurrence after operation. The abdominal US revealed some small cysts in the right liver lobe, whereas serological tests for CEA, CA19-9, CA-125 and AFP were within normal ranges. She was scheduled for a repeat US in 3 mo.

DISCUSSION

BCA is a cystic benign tumor which that originates from intrahepatic or extrahepatic biliary ducts, and it is also called hepatobiliary cystadenoma. BCA represents < 5% of cystic liver disease cases[1]. The cystadenoma is predominantly intrahepatic in origin and rarely seen in extrahepatic bile ducts or the gallbladder[2]. About 90% of BCAs are located intrahepatically, with a slight predilection for the right hepatic lobe. Its size varies from 3-4 cm to a giant cyst of 20-30 cm[3]. Here, we reported a giant intrahepatic BCA located in the left hepatic lobe in a 57-year-old woman.

The etiology of BCA is still unclear, although abnormal embryonic development resulting in ectopic foregut or gonadal epithelium sequestered in the liver has been proposed recently[4]. It seems that BCA has specific epidemiological characteristics. Wang *et al*[5] reported that the majority of intrahepatic BCA cases occurred in women aged ⩽ 60 years, which was consistent with our patient's presentation. However, 11 cases of BCA have been reported in the pediatric population[2,6,7].

Histologically, there are three described subtypes of BCA, based on the underlying type of stroma seen beneath the cuboidal or columnar epithelium. In the first subtype, BCA, microscopically, has a dense, ovarian-like

mesenchymal stroma. This group is the most common and also occurs exclusively in women at a mean age of 40 years. The second subtype has non-ovarian-like stroma and is characterized by the absence of a mesenchymal layer. This group may instead have fibrous, hyaline, or myxoid stroma and is more frequently seen in men with a mean age of 50 years. The third subtype is a cystadenoma that also lacks mesenchymal stroma, but is lined by eosinophilic epithelial cells, which resemble hepatocytes. This group is rare, occurs only in men, and may be a semimalignant histological variant[6]. Our patient lacked a mesenchymal stroma but instead had fibrous stroma, and was classified into the second group.

It is difficult to make an accurate diagnosis of BCA before surgery, and to date no publication has established presenting symptoms that differentiate BCA from other benign or malignant hepatic cystic diseases such as biliary cystadenocarcinoma, hepatic cyst, hydatid cyst, Caroli disease, undifferentiated sarcoma, intraductal papillary mucinous tumor, and hepatocellular carcinoma. Patients may present with abdominal pain, abdominal distention, indigestion, nausea, vomiting, and jaundice, yet patients may be asymptomatic at presentation[8]. On physical examination, an abdominal mass can be identified occasionally. Laboratory results are normal in most patients with BCA, although serum liver enzyme and bilirubinemia levels may be mildly elevated occasionally. Serum AFP, CA19-9, CA-125 and CEA levels are usually within the normal range. It was recently reported that CA19-9 may be elevated in the cystic fluid and contributes to the diagnosis of BCA before surgery[9]. US, CT and MRI play an important role in the diagnosis and antidiastole of the disease. Medical imaging demonstrates intrahepatic cystic lesions with features such as mural nodules, varying wall thickness, papillary projections, and internal septations, which could help to distinguish BCA from other cystic lesions of the liver. On color Doppler US, BCA may appear as a multiloculated anechoic cystic structure with irregular shape and intact membranes. Dense and scattered echogenic dots can be seen in the echo-free area with partitions in between, and solid and papillary echo which is connected to the cystic wall can be seen on the partition wall. Abdominal CT scan may further characterize the multilocular cystic lesion with enhanced, thin internal septations and surrounding normal liver parenchyma, whereas the intraluminal content is usually hypoattenuating. Its fibrous capsule and internal septations are often visible and help distinguish the lesion from a simple cyst. Convex papillae can be seen on the septation, although they are more common in cystadenocarcinoma. MRI may improve tissue characterization because of its high contrast resolution. On MRI, BCA appears as a hypoattenuating lesion on T1WI and hyperintensity cystic fluid on T2WI, but the signal intensity may vary depending on the properties of the cyst fluid[10,11]. On T1WI, the signal intensity may increase with protein concentration. The signal intensity of serous fluid and bile is low. In rare cases, the intensity of serous cystic content can be raised by intracystic hemorrhage and fluid-fluid level can be present. On T2WI, septations with low signal intensity are better visualized in contrast to the high signal intensity of the cystic fluid. If jaundice is present, magnetic resonance cholangiopancreatog-raphy or endoscopic retrogradecholangiopancreatography may be considered to evaluate biliary obstruction. Most commonly, displacement and extrinsic compression of the bile ducts by the tumor is seen, and rarely, communication between the cyst and biliary tree may be observed[12]. With the development of US contrast agent in recent years, contrast-enhanced ultrasonography has become increasingly useful in the diagnosis of liver lesions. BCA manifests as a non-homogeneous rich blood supply cystic masses, the solid part and the wall nodules show strong enhancement in the arterial phase, decreased enhancement in the delayed phase, and equivalent enhancement in the portal phase. Nevertheless, the wall nodules are a little more sharply enhanced in the three phases, making it difficult to differentiate from cystadenocarcinoma. It is well known that US-guided biopsy is the most commonly used method for preoperative pathological diagnosis, and cyst fluid cytology and enzymes can be detected, but carcinomatosis may occur following cyst biopsy[9].

Although imaging is the major diagnostic method for BCA at present, surgery is still the only means of accurate diagnosis. Definitive diagnosis requires histological examination following formal resection, liver transplant, or enucleation[13]. Resection or enucleation with clear margins is the treatment of choice for suspected BCA. Malignant degeneration and recurrence are 30% and 90%, respectively, for incompletely excised lesions[14]. Therefore, patients who only undergo treatment such as aspiration or laparoscopic fenestration would have a poor prognosis. Surgical resection is still necessary when there is diagnostic uncertainty, especially when the patient was complicated with simple or multiple liver cysts. Sometimes, intraoperative frozen sectioning should be performed, in order not to miss a neoplastic condition, such as cystadenocarcinoma. It is important for surgeons to determine the scope of the operation and for patients to receive timely surgery.

Our patient was complicated with polycystic liver disease, which was initially only diagnosed as polycystic liver. This indicates that such cases should be brought to the attention of US physicians and hepatobiliary specialists. In outpatients with diagnosis of hepatic cysts, especially multiple cysts, CT or MRI should be performed when a diagnosis of BCA is suspected.

REFERENCES

1 **Harper RN**, Moore MA, Marr MC, Watts LE, Hutchins PM. Arteriolar rarefaction in the conjunctiva of human essential hypertensives. *Microvasc Res* 1978; **16**: 369-372 [PMID: 748720]

2 **Devaney K**, Goodman ZD, Ishak KG. Hepatobiliary cystadenoma and cystadenocarcinoma. A light microscopic and immunohistochemical study of 70 patients. *Am J Surg Pathol* 1994; **18**: 1078-1091 [PMID: 7943529]

3 **Martínez-González J**, Aicart-Ramos M, Moreira Vicente V. [Hepatic cystadenoma]. *Med Clin* (Barc) 2013; **140**: 520-522 [PMID: 23498841]

4 **Erdogan D**, Busch OR, Rauws EA, van Delden OM, Gouma DJ, van-Gulik TM. Obstructive jaundice due to hepatobiliary cystadenoma or cystadenocarcinoma. *World J Gastroenterol* 2006; **12**: 5735-5738 [PMID: 17007033]

5 **Wang C**, Miao R, Liu H, Du X, Liu L, Lu X, Zhao H. Intrahepatic biliary cystadenoma and cystadenocarcinoma: an experience of 30 cases. *Dig Liver Dis* 2012; **44**: 426-431 [PMID: 22169273 DOI: 10.1016/j.dld.2011.11.007]

6 **Senyüz OF**, Numan F, Eroğlu E, Sarimurat N, Bozkurt P, Cantaşdemir M, Dervişoğlu S. Hepatobiliary mucinous cystadenoma in a child. *J Pediatr Surg* 2004; **39**: E6-E8 [PMID: 14694397]

7 **Tran S**, Berman L, Wadhwani NR, Browne M. Hepatobiliary cystadenoma: a rare pediatric tumor. *Pediatr Surg Int* 2013; **29**: 841-845 [PMID: 23483342 DOI: 10.1007/s00383-013-3290]

8 **Ratti F**, Ferla F, Paganelli M, Cipriani F, Aldrighetti L, Ferla G. Biliary cystadenoma: short- and long-term outcome after radical hepatic resection. *Updates Surg* 2012; **64**: 13-18 [PMID: 22038379 DOI: 10.1007/s13304-011-0117-0]

9 **Koffron A**, Rao S, Ferrario M, Abecassis M. Intrahepatic biliary cystadenoma: role of cyst fluid analysis and surgical management in the laparoscopic era. *Surgery* 2004; **136**: 926-936 [PMID: 15467680]

10 **Buetow PC**, Buck JL, Pantongrag-Brown L, Ros PR, Devaney K, Goodman ZD, Cruess DF. Biliary cystadenoma and cystadenocarcinoma: clinical-imaging-pathologic correlations with emphasis on the importance of ovarian stroma. *Radiology* 1995; **196**: 805-810 [PMID: 7644647]

11 **Gabata T**, Kadoya M, Matsui O, Yamashiro M, Takashima T, Mitchell DG, Nakamura Y, Takeuchi K, Nakanuma Y. Biliary cystadenoma with mesenchymal stroma of the liver: correlation between unusual MR appearance and pathologic findings. *J Magn Reson Imaging* 1998; **8**: 503-504 [PMID: 9562082]

12 **Marrone G**, Maggiore G, Carollo V, Sonzogni A, Luca A. Biliary cystadenoma with bile duct communication depicted on liver-specific contrast agent-enhanced MRI in a child. *Pediatr Radiol* 2011; **41**: 121-124 [PMID: 20585769 DOI: 10.1007/s00247-010-1736-1]

13 **Romagnoli R**, Patrono D, Paraluppi G, David E, Tandoi F, Strignano P, Lupo F, Salizzoni M. Liver transplantation for symptomatic centrohepatic biliary cystadenoma. *Clin Res Hepatol Gastroenterol* 2011; **35**: 408-413 [PMID: 21549659 DOI: 10.1016/j.clinre]

14 **Teoh AY**, Ng SS, Lee KF, Lai PB. Biliary cystadenoma and other complicated cystic lesions of the liver: diagnostic and therapeutic challenges. *World J Surg* 2006; **30**: 1560-1566 [PMID: 16865321]

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Aims and scope

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.00000 35706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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Issue with no volume

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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