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LIVER CANCER

Chinese medicinal compound delisheng has satisfactory anti-tumor activity, and is associated with up-regulation of endostatin in human hepatocellular carcinoma cell line HepG2 in three-dimensional culture

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Abstract

AIM: To investigate the multicellular resistance of human hepatocellular carcinoma HepG2 cells in three-dimensional culture to delisheng, 5-fluorouracil and adriamycin, and the possible molecular mechanisms of delisheng.

METHODS: Human hepatocellular carcinoma HepG2 cells were cultured with a liquid overlay technique. After the formation of multicellular spheroids, morphology was analyzed by phase contrast microscopy, scanning electron microscopy and transmission electron microscopy. Sensitivity of HepG2 cells to delisheng, 5-fluorouracil and adriamycin was investigated by MTT assay in multicelluar spheroids and monolayers. Vascular endothelial growth factor (VEGF) and endostatin expression were analyzed in multicellular spheroids treated with delisheng, 5-fluorouracil, adriamycin and negative control PBS, with immunohistochemical staining.

RESULTS: Multicellular spheroids exhibited structural characteristics somewhat different to those in monolayers. The cells in three-dimensional cell culture turned out to be less sensitive to delisheng, 5-fluorouracil and adriamycin than the cells cultured in monolayer. This showed that delisheng had a satisfactory cells inhibition ratio compared to 5-fluorouracil and adriamycin. Immunohistochemical staining showed that VEGF and endostatin expression was positive during growth as multicellular spheroids, and endostatin expression in spheroids with treatment of delisheng was higher than that with 5-fluorouracil, adriamycin and PBS (139.35 ± 7.83, 159.23 ± 10.34, 162.83 ± 3.47 and 148.48 ± 11.06, $P < 0.05$).

CONCLUSION: Chinese medicine compound delisheng has satisfactory anti-tumor activity in HepG2 cells in three-dimensional culture, and the effects are associated with up-regulation of endostatin.

Key words: Delisheng; Ginseng; Three-dimensional culture; Multicellular resistance; Endostatin

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly malignant tumor with a very high morbidity and mortality, and a poor prognosis. Its incidence is increasing both in Asian countries and in the USA. A majority of HCC patients presents with advanced or unresectable disease. Even for those with resected disease, the recurrence rate can be as high as 50% at 2 years. Despite extensive efforts by many investigators, systemic chemotherapy for HCC has been quite ineffective, as demonstrated by low response rates and no survival benefits[1-4]. With the continuous development of the traditional Chinese medicine industry in recent years, it has been proven that traditional Chinese medicines (TCMs) have a marked effect on treating HCC, with unique advantages, and have gained wide acceptance as a safe, palliative and effective treatment in China[5-9]. Delisheng is a Chinese medicinal compound and is usually used in combination with chemotherapy for HCC. Furthermore, it has been reported that it can improve the clinical symptoms and quality of life, without severe adverse reactions, in patients with late-stage HCC. It is composed of ginseng, milk vetch root, secretion bufonis and Cantharidium. As delisheng is attractive as a natural product for medicinal use, increasing attention is being

paid to its scientific evaluation and its possible molecular mechanisms.

Three-dimensional cell culture has been widely used for studying the various molecular processes and the development of therapy in recent years, for subtle changes in phenotypic expression and biological activity not demonstrated in conventional monolayer culture. In contrast, multicellular spheroids of tumor cells provide an excellent three-dimensional *in vitro* model to facilitate detailed investigations, including the response to various antineoplastic agents and their possible molecular mechanisms, since spheroids mimic the solid tumors more closely than monolayers do[10]. Tumor resistance to anticancer drugs is a real phenomenon, partly because of the so-called multicellular resistance (MCR), and it may be the most important obstacle to cancer treatment[11]. The resistance encountered in cells cultured as spheroids seems to be analogous to the natural resistance observed in patients, so the usage of three-dimensional cell culture may provide a model for studies on the development of anti-cancer drugs.

In this study, cells were cultured with a liquid overlay technique[12,13]. After the formation of multicellular spheroids, morphology was analyzed by phase contrast microscopy, scanning electron microscopy and transmission electron microscopy. Sensitivity of human hepatocellular carcinoma HepG2 cells to delisheng, 5-fluorouracil and adriamycin was investigated by MTT assay in multicelluar spheroids and monolayers. Vascular endothelial growth factor (VEGF) and endostatin expression was analyzed in multicellular spheroids treated with delisheng, 5-fluorouracil, adriamycin and negative control PBS, with immunohistochemical staining.

MATERIALS AND METHODS

Human hepatocellular carcinoma cell line

The human hepatocellular carcinoma cell line used in the present study was HepG2 preserved in The Center of Molecular Biology of Xi'an Jiaotong University.

Monolayer and three-dimensional cell cultures

Each HepG2 cell line was maintained in DMEM (Gibco, USA) medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 U/mL streptomycin in 5% CO_2/95% air at 37℃. Cell cultures were maintained in the exponentially growing state by passaging twice weekly. Exponentially growing cells were harvested in a monolayer cell culture, while for three-dimensional cell culture obtained by liquid overlay technique, a single cell suspension in complete medium was seeded in each culture flask coated with 2% agarose. The conditions for three-dimensional cell culture were exactly the same as for monolayer culture, except for the presence of an agarose layer. After 3 or 4 d incubation, multicellular spheroids were obtained from each culture flask.

Scanning electron microscopy

After 3 or 4 d culture, monolayer cells and multicellular spheroids were observed by phase contrast microscopy. After which, samples were washed with PBS, pH 7.4, and fixed with 2.5% glutaraldehyde for 2 h at 4℃. After three washes in PBS, they were post-fixed with 1.0% osmium tetroxide in PBS for 2 h at 4℃, then washed once in PBS, followed by dehydration with an increasing ethanol series (30, 50, 70, 90 and 100%). The samples were then treated with isoamyl acetate for 10 min, dried to the critical point, and coated with gold. Finally, the samples were observed with a scanning electron microscope (JEOL, JSM-840, Japan).

Transmission electron microscopy

Additional samples were fixed and dehydrated as described for scanning electron microscopy, and embedded in Epon812 epoxy resin. Thin sections were prepared and examined with a transmission electron microscope (HITACHI, H-600, Japan).

MTT assay

The Chinese medicinal compound delisheng was dispensed into a physic liquor to give a clinical dosage of 80 mL (recommended dose is 50-100 mL/d). Delisheng was attenuated with Hanks balanced salts solution, and the final concentration was 12.5, 25, 50, 100 and 200 μL/mL. MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (Sigma, St. Louis, MO, USA) was dissolved in PBS at 5 mg/mL and sterilized by filtration. After treatment with 12.5, 25, 50, 100 or 200 μL/mL delisheng; 6.25×10^{-3}, 12.5×10^{-3}, 25×10^{-3}, 50×10^{-3} and 100×10^{-3} g/L 5-fluorouracil; 0.15625×10^{-3}, 0.3125×10^{-3}, 0.625×10^{-3}, 1.25×10^{-3} and 2.5×10^{-3} g/L adriamycin in monolayer and three-dimensional cell culture for 48 h, the cells were freshly disaggregated by enzymatic dissociation, and the cell number was determined with a hemocytometer. A cell suspension (150 μL) of each sample was added to a 96-well plate. The cell number per well was approximately 2×10^5, Each well was added to stock MTT solution (20 μL, 5 mg/mL). After incubation in the presence of 5% CO_2 and 95% air at 37℃ for 4 h, the supernatant was discarded. DMSO (150 μL) (Sigma) was added to each well and mixed thoroughly to dissolve the dark blue crystals. After 30 min at room temperature to ensure that all crystals were dissolved, the plates were read with a Micro Elisa plate reader at a wavelength of 492 nm. All samples were read five times. The cell inhibition rate was calculated by the following formula: cell inhibition rate (%) = (1-OD of treated cells)/(OD of control cells) × 100%.

Immunohistochemical staining

Antibody staining was performed with multicellular spheroids. The monoclonal anti-VEGF antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used at a dilution of 1:25. The monoclonal anti-endostatin (Santa Cruz Biotechnology) was used at a dilution of 1:25. Spheroids treated with delisheng (25 μL/mL), 5-fluorouracil (40×10^{-3} g/L), adriamycin (1×10^{-3} g/L) and negative control PBS for 48 h were washed in PBS, and fixed in 4% paraformaldehyde in PBS at 4℃ for 1 h, placed in a small tissue processing cassette full of 3%

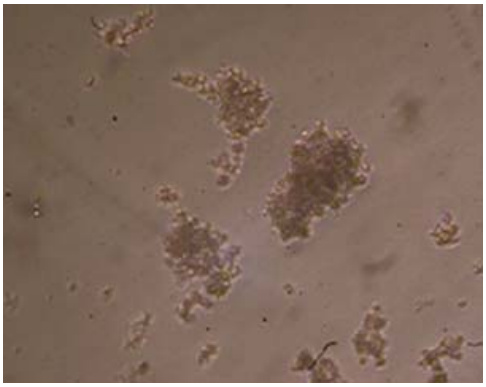

Figure 1 Multicellular spheroids of hepG2 cells observed with the phase contrast microscope (× 100).

Figure 2 Monolayer of hepG2 cells observed with the phase contrast microscope (× 100).

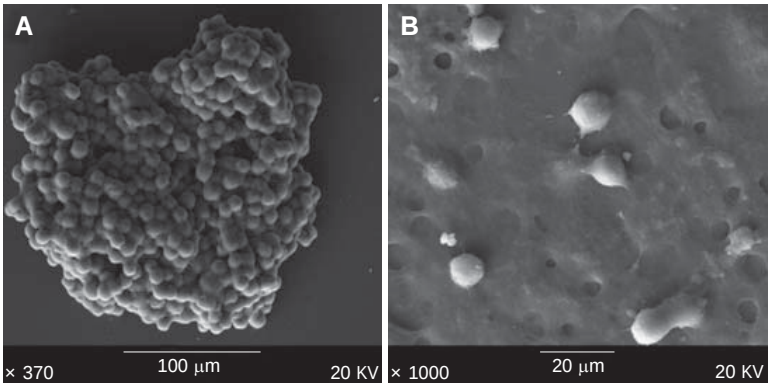

Figure 3 Scanning electron microscopy of hepG2 cells. **A**: The multicellular sphere was irregular with a diameter up to 1.0-2.0 mm, tight cell junctions were observed; **B**: Monolayer cells spread dispersively and cell junctions were hardly observed.

agarose for 1 h, then dehydrated and embedded in paraffin. Four-micrometer sections from the representative blocks were immunohistochemically stained with mouse anti-human monoclonal antibodies against VEGF, and rabbit anti-human monoclonal antibodies against endostatin. Four-micrometer thick sections were deparaffinized, rehydrated and washed in PBS for 15 min, before endogenous peroxidase activity was blocked. Primary antibody was substituted by normal mouse and rabbit serum as a negative control .Then endogenous peroxidase activity was blocked by 30 min incubation in 3% hydrogen peroxide solution. The specimens were washed with PBS, pH 7.5. Non-specific binding was blocked by incubating the slides with normal goat serum in PBS for 15 min at 37℃, then incubated overnight at 4℃ with the primary antibodies. After washing three times with PBS, the sections were incubated with secondary antibody, biotinylated antibodies for 40 min at 37℃. After washing three times with PBS, the sections were immunostained with avidin-biotin complex for 40 min at 37℃. Visualization of the immunoreactions was conducted with 3, 3'-diaminobenzidine (DAB, Sigma, UK) for 5 min. Finally, sections were counterstained with hematoxylin. The degree of the expression of immunohistochemical products was classified into negative (< 10% of cells had a positive reaction) and positive (> 10% of cells had a positive reaction). Simultaneously, VEGF- and endostatin-positive staining particles were quantitatively analyzed by a LeicaQ550cw imaging analysis system (Germany) to determine the mean grey values, the lower mean grey values, and the stronger substrate coloration. The mean grey values are an inverse ratio with the protein expression quantity.

Statistical analysis

Data were reported as means ± SE. The *t* test was used for statistical analysis, $P < 0.05$ was considered statistically significant.

RESULTS

Cell morphology (phase contrast microscopy, scanning and transmission electron microscopy)

Multicellular spheroids and monolayer cells were observed with phase contrast microscopy. The cells were oval spheroids in three-dimensional cell culture (Figures 1 and 2).

Scanning electron microscopy showed that the multicellular spheroids were irregular, with a diameter of up to 200 μm at 3-4 d. The cells were oval spheroids or polyhedrons, with tight cell junctions, compared to monolayer cells that were spread in a dispersed manner and had few cell junctions (Figure 3).

Transmission electron microscopy showed that desmosome and intermediate junctions were observed in three-dimensional cell culture, but such structures were rarely observed in monolayer cell culture. Multicellular spheroids of approximately 200 μm diameter showed no obvious signs of extensive central apoptosis or necrosis,

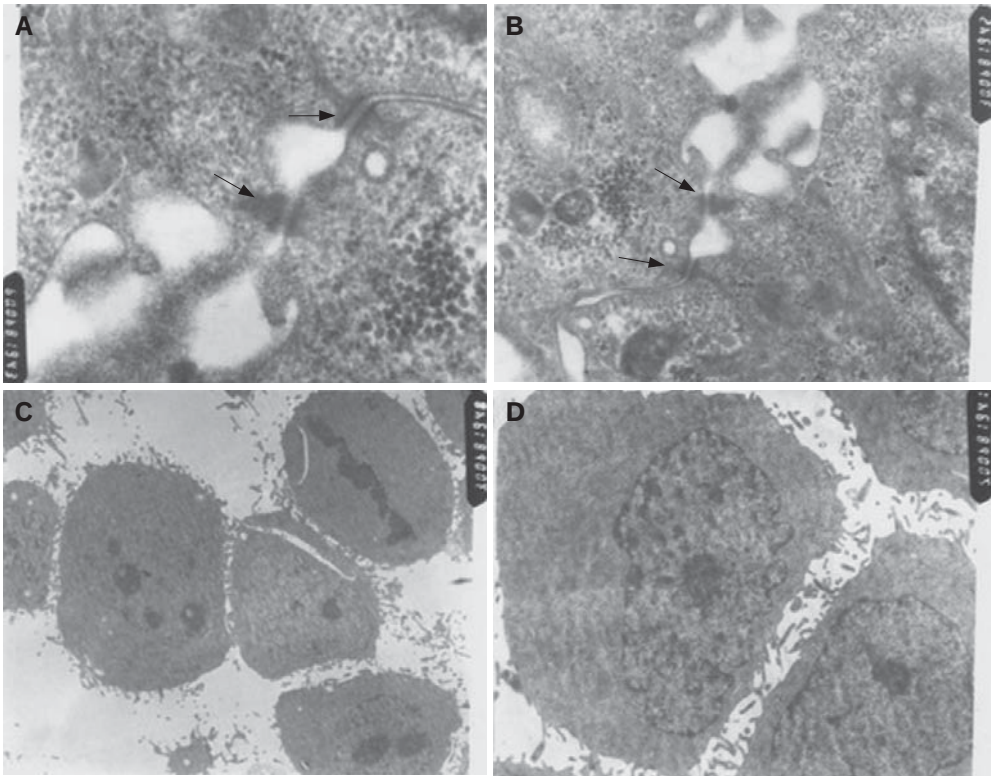

Figure 4 Transmission electron microscopy of hepG2 cells: **A** (× 60 000); **B** (× 30 000): desmosome junctions and intermediate junctions were observed in three-dimensional cell culture; **C** (× 3000); **D** (× 5000): cell junctions were hardly observed in monolayer cells with more microvilli on the surfaces.

Table 1 Test sensitivity of delisheng in 3d and 2d cell culture

Concentration (μL/mL)	Inhibition ratio (%)		*P* value
	3-d	Monolayer	
12.5	11.73 ± 11.58	33.81 ± 10.54	0.014
25	20.94 ± 8.29	37.79 ± 9.55	0.018
50	25.45 ± 5.62	46.01 ± 6.32	0.001
100	36.69 ± 5.37	75.65 ± 10.47	< 0.001
200	43.93 ± 7.81	84.62 ± 3.24	< 0.001

Table 2 Test sensitivity of 5-fluorouracil in 3d and 2d cell culture

Concentration (g/L)	Inhibition ratio (%)		*P* value
	3-d	Monolayer	
6.25×10^{-3}	18.25 ± 7.85	33.89 ± 9.41	0.021
12.50×10^{-3}	17.51 ± 7.28	39.08 ± 7.74	0.002
25.00×10^{-3}	18.18 ± 11.34	43.18 ± 5.92	0.002
50.00×10^{-3}	17.87 ± 10.93	67.72 ± 2.18	< 0.001
100.00×10^{-3}	36.42 ± 10.54	81.17 ± 2.81	< 0.001

although there was some swelling and there were fewer microvilli on the surface of multicellular spheroids compared to monolayer cells (Figure 4).

Response to delisheng, 5-fluorouracil and adriamycin exposure

The sensitivity of monolayer and three-dimensional cell cultures to delisheng, 5-fluorouracil and adriamycin was investigated by MTT assay. The cell inhibition ratio in three-dimensional cell culture treated with delisheng, 5-fluorouracil and adriamycin was lower than that in monolayer culture ($P < 0.01$) (Tables 1-3, Figures 5-7), which indicated the HepG2 cells in three-dimensional culture became resistant to delisheng, 5-fluorouracil and adriamycin. Cell inhibition ratio increased with concentration of delisheng, 5-fluorouracil and adriamycin. The results showed that delisheng had a satisfactory cell inhibition ratio compared to 5-fluorouracil and adriamycin.

Table 3 Test sensitivity of adriamycin in 3d and 2d cell culture

Concentration (g/L)	Inhibition ratio (%)		*P* value
	3-d	Monolayer	
0.15625×10^{-3}	11.40 ± 2.71	10.39 ± 5.07	0.705
0.31250×10^{-3}	12.17 ± 6.49	21.31 ± 2.85	0.02
0.62500×10^{-3}	18.61 ± 9.41	25.69 ± 8.19	0.24
1.25000×10^{-3}	29.45 ± 5.26	41.66 ± 6.52	0.012
2.50000×10^{-3}	38.09 ± 20.71	84.56 ± 7.97	0.002

Data were reported as means ± SE ($n = 5$).

Immunohistochemistry staining

VEGF and endostatin protein expression were confirmed in multicellular spheroids in three-dimensional culture. Immunohistochemical analysis demonstrated that VEGF and endostatin were expressed in the cytoplasm. Endostatin expression in spheroids treated with delisheng was higher than that with 5-fluorouracil, adriamycin and negative control PBS ($P < 0.05$). However, VEGF expression in spheroids treated with delisheng was similar with 5-fluorouracil, adriamycin and PBS ($P > 0.05$) (Tables 4 and 5; Figure 8).

DISCUSSION

HCC is one of the most common types of human

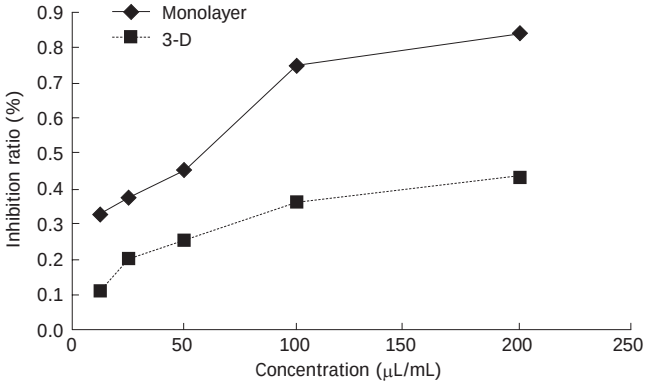

Figure 5 Sensitivity of HepG2 cells to delisheng determined by the MTT assay. After the treatment with 12.5, 25, 50, 100 and 200 μL/mL delisheng for 48 h, the cells in three-dimensional cell culture and monolayer culture were cultured with MTT solution and cell inhibition ratio was determined. The cells in monolayer culture were more sensitive to delisheng ($P < 0.01$).

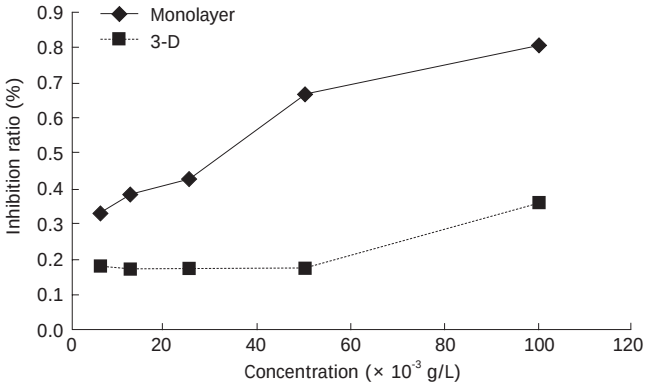

Figure 6 Sensitivity of HepG2 cells to 5-fluorouracil determined by the MTT assay. After the treatment with 6.25, 12.5, 25, 50 and 100 × 10^{-3} g/L 5-fluorouracil for 48 h, the cells in three-dimensional cell culture and monolayer culture were cultured with MTT solution and cell inhibition ratio was determined. The cells in monolayer culture were more sensitive to 5-fluorouracil ($P < 0.01$).

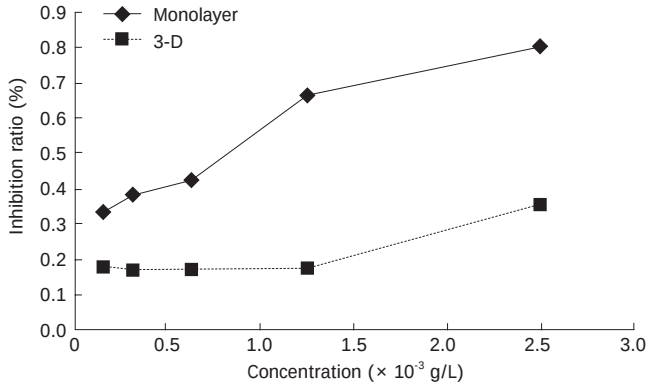

Figure 7 Sensitivity of HepG2 cells to adriamycin determined by the MTT assay. After the treatment with 0.15625, 0.31250, 0.62500, 1.25000, 2.50000 × 10^{-3} g/L adriamycin for 48 h, the cells in-three dimensional cell culture and monolayer culture were cultured with MTT solution and cell inhibition ratio was determined. The cells in monolayer culture were more sensitive to adriamycin ($P < 0.01$).

Table 4 Endostatin expression of multicellular spheroids with treatment of delisheng, 5-fluorouracil, adriamycin and PBS

Drug	Mean grey value
Delisheng	139.35 ± 7.83
5-fluorouracil[a]	159.23 ± 10.34
Adriamycin[c]	162.83 ± 3.47
PBS[e]	148.48 ± 11.06

[a]$P < 0.05$ 5-fluorouracil *vs* delisheng, [c]$P < 0.05$ adriamycin *vs* delisheng, [e]$P < 0.05$ PBS *vs* delisheng.

Table 5 VEGF expression of multicellular spheroids with treatment of delisheng, 5-fluorouracil, adriamycin and PBS

Drug	Mean grey value
Delisheng	188.00 ± 6.33
5-fluorouracil[a]	189.93 ± 16.58
Adriamycin[c]	193.44 ± 5.11
PBS[e]	184.82 ± 13.87

[a]$P > 0.05$ 5-fluorouracil *vs* delisheng, [c]$P > 0.05$ adriamycin *vs* delisheng, [e]$P > 0.05$ PBS *vs* delisheng.

malignancy worldwide. The prognosis in patients with untreated HCC is very poor, with a median survival of 6 mo in patients who receive no specific treatment[14]. Curative therapy such as surgery, liver transplantation[15], or percutaneous treatments benefit only 25% of patients. Systemic chemotherapy has been widely used in an attempt to prolong this short survival time or to provide symptomatic relief[16], but it appears to be less efficient, possibly because it is used when metastases are already present, and the tumor is large and spreading; moreover, anticancer drug resistance is frequent. Tumor resistance to anticancer drugs is a real phenomenon, but the most prevalent mechanism may not correspond to multidrug resistance[17]. Instead, the so-called MCR, first described in 1972 by Durand and Sutherland may be the most important obstacle to cancer treatment. The resistance encountered in cells cultured as spheroids seems to be analogous to the natural resistance observed in patient tumors. Tumor cells often form compact multicellular spheroids when maintained in a three-dimensional culture system. Various changes in molecular expression and even in biological activity have been reported to exist between the three-dimensional and conventional monolayer cultures[18,19].

There are abundant resources in TCMs that have been used clinically for > 5000 years in China and Asia, and increasing attention is being paid to their scientific evaluation. With continuing development of TCM, it has a marked effect on the treatment of several, including tumors, with unique advantages. Delisheng is a common Chinese medicinal compound, whose composition includes ginseng, milk vetch root, secretion bufonis and Cantharidium. Satisfactory effects of delisheng have been reported in patients with late-stage HCC that may improve clinical symptoms and quality of life, without severe adverse reactions. However, the mechanisms responsible for this treatment are unknown. Many kinds of solid tumors *in vivo* and tumor cells in three-dimensional cell culture *in vitro* exhibit intrinsic or acquired resistance to

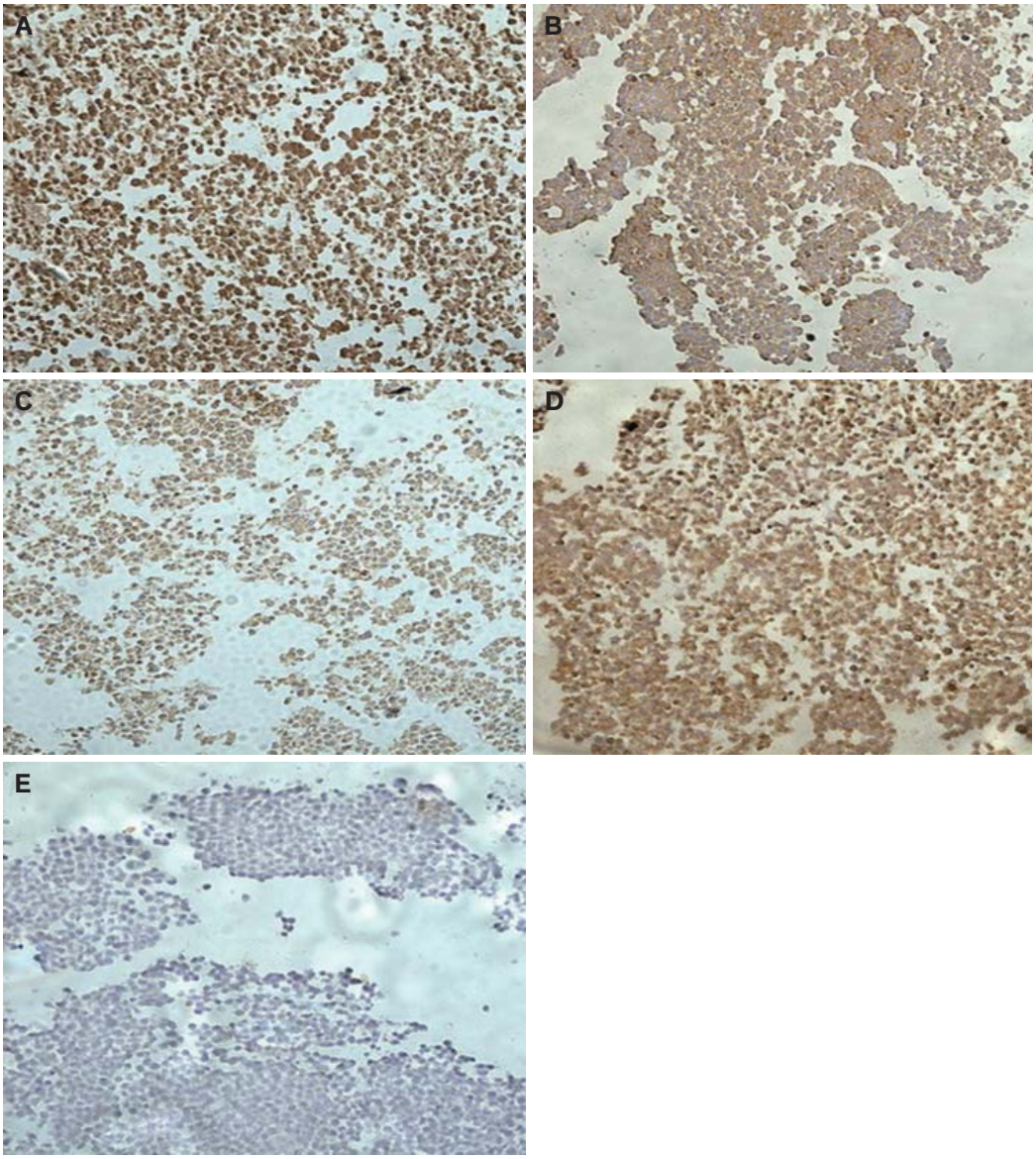

Figure 8 Immunohistochemical staining patterns of formalin-fixed and paraffin-embedded multicellular spheroids of HepG2 cells (× 200) [**A**: delisheng; **B**: adriamycin; **C**: 5-fluorouracil; **D**: negative control PBS; **E**: negative control (Primary antibody was substituted by normal rabbit serum)]. Endostatin expression was confirmed in multicellular spheroids, and the expression of endostatin with treatment of delisheng was higher than that of 5-fluorouracil, adriamycin and PBS ($P < 0.05$).

cytotoxic drugs, which is one of the major obstacles to clinical treatment.

In this study, human HepG2 cells were cultured with a liquid overlay technique to form multicellular spheroids. The results indicated that the cells were oval spheroid or polyhedral, with fewer microvilli on the surface and more desmosome and intermediate junctions, compared to monolayer cells. The cells in three-dimensional culture turned out to be less sensitive to delisheng, 5-fluorouracil and adriamycin than those cultured in monolayer. Some studies have indicated that more cells in multicellular spheroids shift into a quiescent state. However, the majority of conventional cytotoxic anticancer drugs preferentially kill cycling cells. The increase in quiescent cells might result in decreased sensitivity. VEGF and endostatin expression were confirmed in three-dimensional culture. The data indicated that endostatin expression in spheroids treated with delisheng was higher than that with 5-fluorouracil and adriamycin and negative control PBS, and a previous study has shown that delisheng has a satisfactory cell inhibition ratio compared to 5-fluorouracil and adriamycin. This suggests that the satisfactory effects of delisheng on HCC were associated with the up-regulation of endostatin. As we know, one of the components of delisheng, ginseng, has some antiangiogenic activity. Recent studies have reported that ginseng extract exerts anti-tumor activity through its effect on the vascular system; furthermore, some investigators have suggested that ginsenoside Rg3, a saponin extracted from ginseng, alone or combined with cyclophosphamide (CTX), inhibits growth and angiogenesis of ovarian cancer by decreasing the microvessel density (MVD value) and VEGF expression[20-23]. Endostatin as an angiogenesis inhibitor has been shown to inhibit VEGF-induced endothelial cell migration *in vitro*, and to have anti-tumor activity *in vivo*[24,25]. Some investigators have studied tumor growth in transgenic mice overproducing endostatin specifically in the endothelial cells (a 1.6-fold increase in the circulating levels), and found that tumor growth was 3-fold slower than in wild-type mice[26-30]. Therefore, we think that the endostatin up-regulation induced by delisheng in our experiment was possibly due to the antiagiogenic activity of its ginseng component, and this may explain why delisheng has satisfactory anti-tumor

activity too. Although major emphasis has been placed on the down-regulation of VEGF, the potential role of endostatin increase induced by ginseng as an endogenous inhibitor of angiogenesis in tumor growth inhibition can not be ignored.

Further understanding of the mechanisms involved in the activity of delisheng will help in the development of new approaches to therapy of HCC. The satisfactory activity of delisheng on HCC was associated with ginseng up-regulation of endostatin expression.

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COMMENTS

Background

HCC is a highly malignant tumor with a very high morbidity and mortality. Despite extensive efforts by many investigators, systemic chemotherapy for HCC has been quite ineffective. Delisheng is a Chinese medicinal compound and is often used in conjunction with chemotherapy for HCC, with satisfactory results. Our work tried to establish the mechanisms for these effects of delisheng on HCC. Three-dimensional cell culture has been widely used for studying the various molecular processes, since spheroids mimic solid tumors more closely than monolayers do, so the use of three-dimensional culture provides a model for the development of anti-cancer drugs. In this study, cells were cultured with a liquid overlay technique. After the formation of multicellular spheroids, we used the model to perform our experiments.

Research frontiers

With the continuous development of TCM in recent years, it is been demonstrated that it can have a marked effect on treating HCC, with unique advantages, and it has gained wide acceptance as a safe, palliative and effective treatment in China. Delisheng is a Chinese medicinal compound and is often used in conjunction with chemotherapy for HCC. Furthermore, it has been reported in patients with late-stage HCC that delisheng may improve the clinical symptoms and quality of life, without severe adverse reactions. As delisheng is attractive as a natural product for medicinal use, increasing attention is being paid to its scientific evaluation and its possible molecular mechanisms. Three-dimensional cell culture has been widely used for studying the various molecular processes and development of therapy in recent years, as it detects subtle changes in phenotypic expression and biological activity not seen in conventional monolayer culture. In contrast, multicellular spheroids of tumor cells provide an excellent three-dimensional *in vitro* model to facilitate detailed investigations, including the response to various antineoplastic agents and their possible molecular mechanisms, since spheroids mimic solid tumors more closely than monolayers do. The resistance encountered in cells cultured as spheroids seems to be analogous to the natural resistance observed in patient tumors, so the usage of three-dimensional cell culture may provide a model for developing anti-cancer drugs.

Innovations and breakthroughs

We used three-dimensional cell culture to study a Chinese medicine and its anti-cancer effects. We showed that delisheng had satisfactory anti-cancer effects on HCC, and these were associated with the up-regulation of endostatin. This was possible because of the presence of ginseng in delisheng.

Applications

We confirmed that three-dimensional cell culture was suitable for the study of a traditional Chinese medicine, and this may help other researchers to find a better model for drug development. We also found that delisheng had satisfactory anti-cancer effects on HCC, and these were associated with the up-regulation of endostatin. This was made possible by one of delisheng's components, ginseng, and this may provide a new method of therapy for HCC.

Terminology

Three-dimensional cell culture: this has been widely used for studying the various molecular processes and development of therapy in recent years, because it can detect subtle changes in phenotypic expression and biological activity not seen in conventional monolayer culture. This is because spheroids mimic solid tumors more closely than monolayers do. Delisheng: a Chinese medicinal compound that is often used in conjunction with chemotherapy for HCC. Furthermore, it has been reported in patients with late-stage HCC that it can improve clinical symptoms and quality of life, without severe adverse reactions. Its composition includes ginseng, milk vetch root, secretion bufonis and Cantharidium.

Peer review

The article provides a new model to study TCM, and explains the test outcome rationally; furthermore, it introduces the Chinese medicinal compound delisheng and indicates its further applications.

REFERENCES

1 **Okuda K**. Hepatocellular carcinoma. *J Hepatol* 2000; **32**: 225-237
2 **Rougier P**, Mitry E, Barbare JC, Taieb J. Hepatocellular carcinoma (HCC): an update. *Semin Oncol* 2007; **34**: S12-S20
3 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917
4 **Llovet JM**, Fuster J, Bruix J. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. *Liver Transpl* 2004; **10**: S115-S120
5 **Chen RC**, Su JH, Ouyang GL, Cai KX, Li JQ, Xie XG. Induction of differentiation in human hepatocarcinoma cells by isoverbascoside. *Planta Med* 2002; **68**: 370-372
6 **Efferth T**, Davey M, Olbrich A, Rucker G, Gebhart E, Davey R. Activity of drugs from traditional Chinese medicine toward sensitive and MDR1- or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukemia cells. *Blood Cells Mol Dis* 2002; **28**: 160-168
7 **Mabed M**, El-Helw L, Shamaa S. Phase II study of viscum fraxini-2 in patients with advanced hepatocellular carcinoma. *Br J Cancer* 2004; **90**: 65-69
8 **Jang M**, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997; **275**: 218-220
9 **Cheng JT**. Review: drug therapy in Chinese traditional medicine. *J Clin Pharmacol* 2000; **40**: 445-450
10 **Desoize B**, Jardillier J. Multicellular resistance: a paradigm for clinical resistance? *Crit Rev Oncol Hematol* 2000; **36**: 193-207
11 **Hoffman RM**. The three-dimensional question: can clinically relevant tumor drug resistance be measured in vitro? *Cancer Metastasis Rev* 1994; **13**: 169-173
12 **O'Connor KC**, Venczel MZ. Predicting aggregation kinetics of DU 145 prostate cancer cells in liquid-overlay culture. *Biotechnol Lett* 2005; **27**: 1663-1668
13 **Carlsson J**, Yuhas JM. Liquid-overlay culture of cellular spheroids. *Recent Results Cancer Res* 1984; **95**: 1-23
14 **Okuda K**, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985; **56**: 918-928
15 **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
16 **Leung TW**, Johnson PJ. Systemic therapy for hepatocellular carcinoma. *Semin Oncol* 2001; **28**: 514-520
17 **Perez-Tomas R**. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. *Curr Med Chem* 2006; **13**: 1859-1876
18 **O'Brien LE**, Yu W, Tang K, Jou TS, Zegers MM, Mostov KE. Morphological and biochemical analysis of Rac1 in three-dimensional epithelial cell cultures. *Methods Enzymol* 2006;

406: 676-691
19 **Hamilton G**. Multicellular spheroids as an in vitro tumor model. *Cancer Lett* 1998; **131**: 29-34
20 **Liu CX**, Xiao PG. Recent advances on ginseng research in China. *J Ethnopharmacol* 1992; **36**: 27-38
21 **Xu TM**, Xin Y, Cui MH, Jiang X, Gu LP. Inhibitory effect of ginsenoside Rg3 combined with cyclophosphamide on growth and angiogenesis of ovarian cancer. *Chin Med J* (Engl) 2007; **120**: 584-588
22 **Folkman J**. Angiogenesis. *Annu Rev Med* 2006; **57**: 1-18
23 **Sengupta S**, Toh SA, Sellers LA, Skepper JN, Koolwijk P, Leung HW, Yeung HW, Wong RN, Sasisekharan R, Fan TP. Modulating angiogenesis: the yin and the yang in ginseng. *Circulation* 2004; **110**: 1219-1225
24 **O'Reilly MS**, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997; **88**: 277-285
25 **Shi W**, Teschendorf C, Muzyczka N, Siemann DW. Adeno-associated virus-mediated gene transfer of endostatin inhibits angiogenesis and tumor growth in vivo. *Cancer Gene Ther* 2002; **9**: 513-521
26 **Sasaki T**, Fukai N, Mann K, Gohring W, Olsen BR, Timpl R. Structure, function and tissue forms of the C-terminal globular domain of collagen XVIII containing the angiogenesis inhibitor endostatin. *EMBO J* 1998; **17**: 4249-4256
27 **Carmeliet P**, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000; **407**: 249-257
28 **Hood JD**, Bednarski M, Frausto R, Guccione S, Reisfeld RA, Xiang R, Cheresh DA. Tumor regression by targeted gene delivery to the neovasculature. *Science* 2002; **296**: 2404-2407
29 **Yamaguchi N**, Anand-Apte B, Lee M, Sasaki T, Fukai N, Shapiro R, Que I, Lowik C, Timpl R, Olsen BR. Endostatin inhibits VEGF-induced endothelial cell migration and tumor growth independently of zinc binding. *EMBO J* 1999; **18**: 4414-4423
30 **Sund M**, Hamano Y, Sugimoto H, Sudhakar A, Soubasakos M, Yerramalla U, Benjamin LE, Lawler J, Kieran M, Shah A, Kalluri R. Function of endogenous inhibitors of angiogenesis as endothelium-specific tumor suppressors. *Proc Natl Acad Sci USA* 2005; **102**: 2934-2939

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EDITORIAL

Genetic epidemiology of primary sclerosing cholangitis

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Abstract

The aetiology of primary sclerosing cholangitis (PSC) is not known. A more than 80-fold increased risk of PSC among first-degree relatives emphasizes the importance of genetic factors. Genetic associations within the human leukocyte antigen (HLA) complex on chromosome 6p21 were detected in PSC 25 years ago. Subsequent studies have substantiated beyond doubt that one or more genetic variants located within this genetic region are important. The true identities of these variants, however, remain to be identified. Several candidate genes at other chromosomal loci have also been investigated. However, according to strict criteria for what may be denominated a susceptibility gene in complex diseases, no such gene exists for PSC today. This review summarises present knowledge on the genetic susceptibility to PSC, as well as genetic associations with disease progression and clinical subsets of particular interest (inflammatory bowel disease and cholangiocarcinoma).

Key words: Primary sclerosing cholangitis; Genetic associations; Human leukocyte antigens; Cholangiocarcinoma; Inflammatory bowel disease

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic inflammatory condition of unknown aetiology, characterised by progressive strictures of the intra- and extrahepatic bile ducts and eventually liver cirrhosis and liver failure[1,2]. No effective medical treatment is currently available[3,4], and PSC is the major indication for liver transplantation in the Scandinavian countries as well as the fifth leading indication for liver transplantation in the United States[5,6]. Population-based studies of disease frequency are available from Norway, Great Britain and The United States[7-9], and indicate comparable incidence (0.9-1.3 per 100 000/year) and prevalence (8.5-14.2 per 100 000) rates for these populations. The prevalence of PSC is probably lower in Southern European and Asian populations[10]. In contrast to the female predominance of many autoimmune diseases, approximately 2/3 of the PSC patients are male[11]. Affected individuals are young (less than 40 years at time of diagnosis), and median survival from time of diagnosis by cholangiography to death or liver transplantation is approximately 12 years[11].

Up to 80% of the PSC patients of Northern European origin have concurrent inflammatory bowel disease (IBD)[10]. The frequency in Southern Europe and Asia is lower (around 50% and 35%, respectively)[12-14]. According to standard criteria[15], the IBD phenotype in PSC has mainly been classified as ulcerative colitis (UC), although an association with colonic Crohn's disease also exists[16,17]. The increased frequency of a variety of other autoimmune diseases (e.g. type 1 diabetes) among patients with PSC does not seem related to the increase in IBD[18]. There is also an increased risk of cancer among the patients with PSC, not only cholangiocarcinoma of the biliary tract (approximately 13%-14% in Scandinavia)[19,20], but also other gastrointestinal malignancies (i.e. pancreatic and colorectal cancer)[19]. The diagnosis of cholangiocarcinoma is difficult because the cholangiographic changes may look similar to those found in PSC without cholangiocarcinoma[21]. As a result, the cancer is often recognised at an advanced stage when treatment by liver transplantation does not improve survival[22].

Smoking is the only environmental factor known to influence PSC susceptibility and is associated with a reduced risk of the disease[23]. Several genetic risk factors, however, have been repeatedly described throughout the 25 years since they were first detected[24,25]. The present editorial aims to summarise present knowledge on statistical associations between genetic variants and risk of PSC or particular characteristics of PSC. In genetic epidemiology, disease characteristics under study are called phenotypes. Etymologically, the pheno-prefix refers to "visible" or "evident". Phenotypes, also referred to as traits, may be dichotomous (e.g. PSC/healthy) or quantitative (e.g. the level of alkaline phosphatase in a blood sample from a PSC patient). The clinical definition of a disease is primarily made to decide whether a

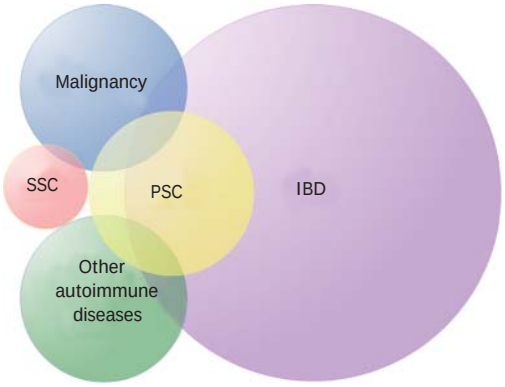

Figure 1 Primary sclerosing cholangitis (PSC) is a patchwork of different phenotypes in addition to the bile duct involvement. Most important are inflammatory bowel disease (IBD), malignancy and other autoimmune diseases. PSC is distinct from secondary sclerosing cholangitis (SSC).

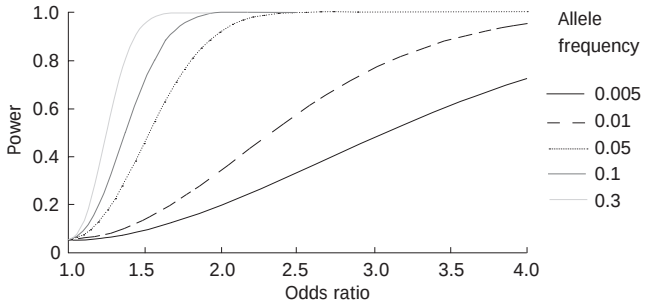

Figure 2 Statistical power (α = 0.05) for different odds ratios and allele frequencies in a study of 365 patients and 365 controls, i.e. the number of alleles in each group is 2 n = 730.

particular treatment or follow-up may be indicated for a patient or not. This practical aspect means that PSC as a clinical "diagnosis" does not necessarily equal the ideal "phenotype" for genetic association studies. The disease phenotype in such studies should be as homogeneous as possible, simply because the presence of irrelevant phenotypes in a study population will reduce the strength of effects to be identified. The clinical phenotype of PSC is compound (Figure 1).

In other diseases, susceptibility genes have been identified through genome-wide linkage scans followed by fine-mapping[26-28]. In PSC, the lack of families with affected sibling pairs has not allowed such studies to position susceptibility loci[28]. The search for PSC susceptibility genes has thus focused on plausible candidates with regard to function[25]. As a general basis for interpreting candidate gene association studies, an introduction to important concepts of such studies will be given, followed by a presentation and discussion of studies performed in PSC. We searched PubMed for relevant articles published up until the end of April 2007. We have also reviewed the reference lists of identified articles, as well as the reference lists of major immunogenetic- and hepatology conferences held over the last 2 years.

GENETIC CONSIDERATIONS

In genetic terms, PSC is considered a complex trait, meaning that polymorphisms in several genes along with environmental factors are required for disease development[27]. Heritability for a disease is measured by (a) concordance rates in monozygotic versus dizygotic twins and (b) relative risk in siblings of a patient (λ_s = prevalence among siblings divided by the general population prevalence). For monogenic disorders, λ_s ranges from several hundreds to several thousands, whereas values in complex traits are usually below 100. A strong genetic contribution to overall risk of PSC is supported by λ_s values of approximately 100[29], as compared with values of 15-35 for Crohn's disease and 6-9 for UC[30].

Polymorphisms are genetic variants that have arisen from mutational events in DNA[31]. Conventionally, to be denominated a polymorphism, a mutant variant should occur at a frequency of > 0.01 in the general population. A particular nucleotide (or nucleotide sequence) at a polymorphism is defined as an allele. The combination of alleles on the two chromosomes is termed the genotype of the individual at that position. A distinct combination of two or more alleles of polymorphisms that occur together on the same chromosome is defined as a haplotype.

When a mutation arises in a chromosomal region, it does so on a background of particular DNA variants that are already present in the population, i.e. the mutation is linked to these surrounding alleles by the integrity of the DNA molecule. Over time, recombination tends to separate a mutant allele from the alleles of the surrounding DNA. At the population level, the positive association that remains between particular alleles at linked polymorphisms is called linkage disequilibrium (LD), meaning that these alleles occur more frequently together than would be expected from their population frequencies. Recombination ultimately leads to loss of LD unless there is a selective advantage of particular allele combinations.

The relationship between disease phenotype and three of the genetic concepts described (polymorphisms, alleles and haplotypes), is the subject of *genetic association studies*. That is, the aim of genetic epidemiology is to identify alleles (or in diploid terms, genotypes) of polymorphisms that are associated with an increase or decrease in risk of disease or a particular characteristic of a disease. The advantage of LD is that all polymorphisms in a genetic region do not have to be genotyped to detect an association. This is because the causative variant will reside on the same haplotypes as other polymorphisms and can be indirectly detected by typing for these. The disadvantage of LD is that it may be almost impossible to determine which of a series of alleles in LD on a haplotype that is actually the causative variant. Most of the genetic variation (> 99%) in the human genome is believed to be without any phenotypic consequence[32].

STATISTICAL CONSIDERATIONS

Because of the low prevalence, a major limiting factor for statistical power in studies of PSC susceptibility genes is sample size. Figure 2 illustrates the statistical power as a function of the effect size (odds ratio; OR) and allele

frequency of a genetic variant for studies performed in the largest PSC population in which studies have been performed so far (n = 365)[33]. Two issues require mentioning. First, very weak effects (OR ≈ 1.0-1.3) are likely to be missed, even for populations of this size. Second, rare variants of importance for PSC susceptibility (allele frequency < 0.01) are likely to be missed unless the OR of the variant is very high (or low; ORs < 1 were not plotted for clarity).

An important controversy regarding the prospects of mapping the genetic predisposition to complex diseases is not related to statistical power, but the possible complexity of allelic variation at a susceptibility locus. Supporters of the "common-disease/common-variant" hypothesis argue that common diseases arise due to polymorphisms that are common (i.e. allele frequency > 0.10[34]) in the background population. Supporters of the "multiple rare variants" hypothesis point to the complexity observed at susceptibility loci in monogenic disorders, where multiple rare alleles define a similar phenotype (e.g. the hundreds of disease causing alleles at the cystic fibrosis transmembrane-conductance regulator locus)[35]. Possibly, susceptibility genes in complex diseases that are defined by multiple rare variants cannot be identified using regular LD based approaches[36]. Although PSC is relatively rare, the main HLA haplotypes that confer risk are relatively common (e.g. the frequency of the PSC associated ancestral HLA haplotype 8.1 is > 0.10 in Scandinavia[37]).

The abundance of false positive genetic association studies (i.e. type I statistical errors) represents a problem of legitimacy for this type of study design[38]. Simply using a P-value < 0.05 as "evidence" to distinguish between a "positive" and "negative" finding in these studies can be questioned[39]. The problem is partly related to the many statistical tests performed in these studies. The so-called Bonferroni correction (multiplying P-values with the number of comparisons that have been performed) is the most widely accepted strategy to account for this problem.

The Bonferroni approach has limitations. Due to the many tests that are theoretically possible throughout the genome, it can be argued that conservative significance levels of 10^{-5} or even 10^{-8} should be used for all tests[38,40]. Achieving such significance levels would require patient collections simply not available for rare diseases like PSC. The most recent proposal is that so-called permutation testing (in Latin, "permutare" means "change completely") within a dataset is the preferable strategy to take account of multiple testing[41]. In permutation tests, case/control assignment is shuffled randomly using a computer and tests are run over and over again to count how often the permuted dataset achieves the effect observed in the correctly ordered dataset. If the permuted dataset achieves an effect equal to or stronger than that observed in the original dataset in 500 out of 10 000 analyses, this means that the probability of a type I error for a finding is 5%.

The problem of statistical significance in genetic association studies philosophically relates to the problem of causality for which criteria relevant to modern medicine were proposed by Sir Austen Bradford Hill in a classic essay in 1965[42]. These criteria point to factors in addition to the probability from statistical association tests (e.g. biological plausibility) that are required for a causal relationship to be established. This is also argued for in so-called Bayesian statistics, where the prior probability of a genetic variant to be associated (e.g. non-synonymous polymorphism in a gene which function is relevant to the disease phenotype), is accounted for when deciding on the posterior probability of whether or not a finding is valid[38]. In sum, circumstantial evidence (from functional studies or mouse models) is required to support findings if a genetic variant should be considered causative in terms of contributing to a disease phenotype[28], whatever the statistical evidence is available.

THE HLA COMPLEX AND GENETIC ASSOCIATIONS OBSERVED IN PSC

The HLA complex stretches across 7.6 million base pairs (bp) of DNA on the short arm of chromosome 6 and contains 252 expressed protein-coding genes, of which 28% are potentially related to immunological functions[43]. Throughout evolution of this genetic region[44], duplications have led to several gene clusters containing genes of similar function (Figure 3)[43]. HLA class I molecules (i.e. HLA-A, -B and -C) are expressed on all nucleated cells in the body and present intracellular/endogenous antigens to $CD8^+$ T-lymphocytes. HLA class I molecules also serve as ligands for inhibitory killer immunoglobulin-like receptors (KIRs) on natural killer (NK) cells and γδ T-lymphocytes[45,46]. HLA class II molecules are expressed on antigen presenting cells (e.g. macrophages and dendritic cells) and present extracellular/exogenous antigens to $CD4^+$ T-lymphocytes[45].

Sequence-based HLA-nomenclature was established in 1987[45]. The locus name is followed by an asterisk and two pairs of digits. The first pair of digits denominates the main type and is often similar to the serological type (e.g. DRB1*03 is the same as serological DR3, but DRB1*13 is only one of the DR6 alleles). The second pair of digits denominates the subtype (e.g. DRB1*0301 and DRB1*1301). Further definition is possible, since null alleles are suffixed by "N", and polymorphisms that do not alter the amino acid sequence of the peptide binding groove give rise to the fifth, sixth and seventh digits. In result, a complete sequence-based HLA allele name represents the haplotype of all alleles at all polymorphisms within the HLA gene at that chromosome.

LD between alleles at the HLA class I and II loci defines ancestral HLA haplotypes (AHs) and are named after which HLA-B allele they contain (e.g. the most common haplotype with HLA-B*08 is called AH8.1)[44]. Alleles of other genes are in LD with these ancestral haplotypes, and the co-occurrence of particular alleles across the entire HLA complex on one chromosome is called an extended HLA haplotype[47]. At the population level, the degree of conservation varies between different extended HLA haplotypes[48]. As examples of this phenomenon, an extended HLA haplotype with the HLA-B*08 and DRB1*0301 alleles (i.e. the AH8.1) is remarkably conserved in the Northern European population, whereas haplotypes carrying DRB1*04 alleles

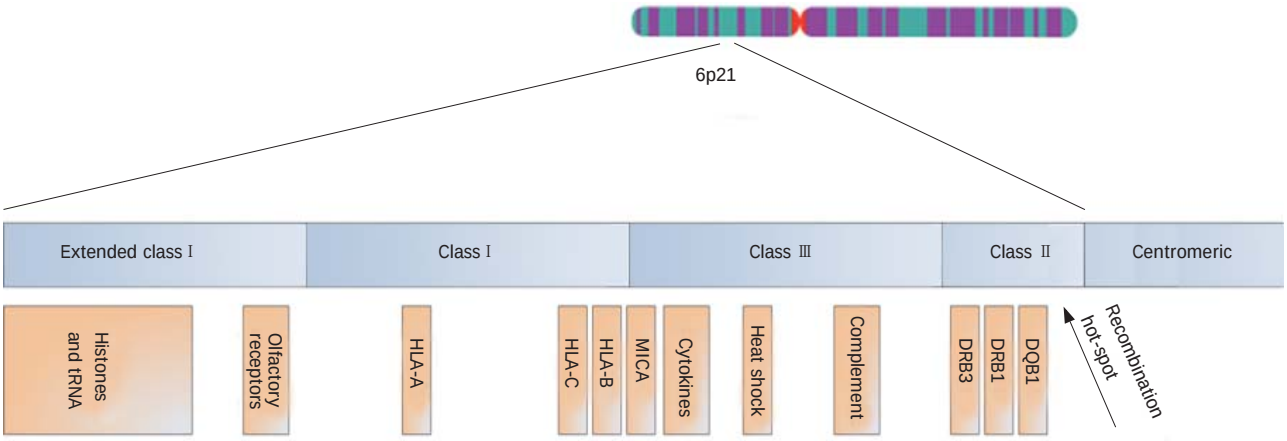

Figure 3 Schematic outline of the HLA complex on chromosome 6. Distances are arbitrary. By convention, the extended HLA complex stretches from the centromeric border of the HLA class Ⅱ loci (HLA-DP) to the telomeric limit of the histone gene cluster more than 4 million bp from HLA-A[43,120]. Centromeric to the HLA-DQ loci, a region with intense recombination can be found ("recombination hot-spot")[132].

are considerably less conserved and may not even qualify for the denomination "extended haplotypes"[49].

A HLA association in PSC was first identified for HLA-B8 (i.e. HLA-B*0801) and DR3 (i.e. DRB1*0301)[24,50]. Later studies have verified that PSC associations exist also for the other alleles of the AH8.1 (the HLA-A1 allele[51], the HLA-C7 allele[52], the major histocompatibility complex class I chain-related A (*MICA*) *008/5.1 allele[53,54], and the tumour necrosis factor alpha (*TNF*α) promoter -308 A allele[55,56]). This haplotype is associated with a wide range of autoimmune diseases[57,58]. A cross-European study (Norway, Sweden, Great Britain, Italy and Spain) concluded that a consistent, positive HLA class Ⅱ association in PSC probably exists also for a haplotype that carries the DR6 (i.e. DRB1*1301) allele[37]. In individuals negative for DR3 and DR6, an association with haplotypes that carry the DR2 (i.e. DRB1*1501) allele can be found. Negative associations with HLA class Ⅱ alleles have been reported for the DR4, DR7 and DR11 alleles[37,59,60], although primarily in populations of Northern European origin[56]. In Southern Europe, the picture is even more complex, since the DR4 allele seems to be consistent in LD with a predisposing variant in Italy[37,56], whereas a protective effect is noted in Spain[37].

Due to strong LD, an important question in HLA genetics is whether genetic associations are due to variation in the HLA class I or Ⅱ genes (meaning that they arise because the patients are able to present particular antigens to the T-cell receptor)[61], or due to variation in neighbouring genes[62]. There is some degree of amino acid sequence similarity between several of the PSC associated HLA class Ⅱ polypeptide variants[59,63]. However, no consistency has been found regarding these similarities[59]. The proposal of leucine at position 38 of the DRβ polypeptide as a critical determinant for PSC susceptibility relies heavily on the strong DRB3*0101 association in Northern European populations[63]. An early suggestion that a common denominator between haplotypes with the DRB1*0301 and DRB1*1301 alleles could be the DRB3*0101 allele (serologically DRw52a) was later withdrawn[64,65]. Another study found that the DRB1*1301-DRB3*0202 haplotype association is as strong as the DRB1*1301-DRB3*0101 association[37]. Taken together, the most interesting proposal of a single amino acid position in defining risk of PSC may rather relate to a protective effect in carriers of proline at position 55 of the DQβ polypeptide, which is common for DQ3 alleles known to be in LD with the protective DR4, DR7 and DR11 alleles[59]. However, no consistent risk allele is defined by this position[59], and to what extent the HLA class Ⅱ molecules are of primary importance in the PSC pathogenesis should probably not be concluded based on present evidence.

The PSC-associated MICA*008/5.1 allele has been proposed as a common denominator between the PSC-associated A*01-C*07-B*08-DRB1*0301-DQB1*0201 and A*03-C*07-B*07-DRB1*1501-DQB1*0602 haplotypes[53,59]. MICA functions as a ligand for the activating NKG2D receptor on NK cells[66]. It was recently recognised that the two risk haplotypes in question share alleles not only at *MICA*, but also at the neighbouring *HLA-B* and *-C* loci, when these are defined according to the KIR binding properties of the HLA class I molecules[67]. The PSC-associated *HLA-B* and *-C* KIR ligand genotypes may result in decreased inhibition of NK cells and several subsets of T-lymphocytes that express KIRs[46,68]. Such combinations of KIR and HLA class I ligand variants have been shown to increase susceptibility to other autoimmune diseases[46]. How the PSC-associated MICA*008/5.1 allele may cause disease is not known. This allele is also associated with an increased risk of other autoimmune conditions[69,70], and may thus also result in an increased activity of cells expressing the NKG2D receptor, acting in synergy with the loss of inhibition resulting from the PSC associated HLA class I ligand genotypes. The fact that the MICA 5.1 allele was recently shown to confer protection against cholangiocarcinoma is in line with an activating effect[71]. Some studies report an increased frequency of NK cells in the portal infiltrate of patients with PSC when compared with other liver diseases[72,73], and also in the intestinal mucosa of patients with PSC without IBD compared with IBD patients without liver

Table 1 Candidate gene studies performed in PSC

Gene	Chromosome	N (PSC)	Primary finding	Reference	Replication finding	Reference
IL-1	2q	40	Negative	[76]	Negative	[77]
IL-10	1q	96	Negative	[77]	Negative	[55]
MMP1	11q	165	Negative	[78]	NA	-
MMP3	11q	111	Positive	[79]	Negative	[78]
CCR5	3p	71	Positive	[80]	Negative	[33]
ICAM-1	19p	104	Positive	[81]	Negative	[82]
CFTR	7q	29	Negative	[83]	Negative	[84,85]
MDR3	7q	37	Negative	[86]	Negative	[87]
BSEP	2q	37	Negative	[86]	NA	-
AIRE	21q	60	Negative	[88]	NA	-
NRAMP1	2q	40	Negative	[89]	NA	-
CTLA4	2q	144	Negative	[90]	NA	-
FOXP3	X	195	Negative	[91]	NA	-

Interleukin-1 and -10 (IL-1 and -10), MMP1 and 3 (matrix metalloproteinase 1 and 3), CCR5 (chemokine receptor 5), ICAM-1 (intercellular adhesion molecule 1), CFTR (cystic fibrosis transmembrane conductance regulator), MDR3 (multidrug resistance gene 3), BSEP (bile salt export pump), AIRE (autoimmune regulator), NRAMP1 (natural resistance-associated macrophage protein 1), CTLA4 (cytotoxic T-lymphocyte-associated protein 4), FOXP3 (forkhead box P3). NA: Not available.

disease[74]. Taken together with the genetic findings in this region of the HLA complex (Figure 3), further studies on the role of these cells in PSC seem warranted.

In sum, the HLA association in PSC is likely to be complex. Multiple risk variants may exist[25], some of which may be associated not only with PSC, but autoimmunity in general.

PSC ASSOCIATIONS WITH POLYMORPHISMS IN GENES OUTSIDE THE HLA COMPLEX

Summarising the published genetic association studies in PSC, it seems proven beyond doubt that one or more genetic variants located within the HLA complex are important. The true identities of these variants, as discussed above, are not known. The situation is even less clear with regard to other susceptibility loci. Given the large number of protein coding genes in the human genome (25-35 000)[32], selecting candidate genes for association studies is an extremely difficult task. According to strict criteria for what may be denominated a susceptibility gene in complex diseases (consistent statistical evidence, functional consequence of identified mutation, relevant tissue expression, *etc.*)[28], no such gene exists for PSC. A summary of studies performed is given in Table 1. So far, most attention has been given to genes known to be of importance in other autoimmune diseases. The association between PSC and IBD has also inspired some of the studies, as well as the observation of PSC-like changes in cystic fibrosis[75].

Two of the negative findings are of particular interest and will be discussed in greater detail. First, studies in limited populations ($n < 50$) have pointed to a non-significant increase of particular multidrug resistance gene 3 (*MDR3*) variants among PSC patients as compared with healthy controls[86,87]. Knock-out mice for this phospholipid transporter gene (called *mdr2* in mice) spontaneously develop hepatic lesions resembling PSC[92], possibly due to loss of protection of the biliary epithelium from toxic bile acids. Second, it cannot be formally ruled out that the 32 bp deletion of the chemokine receptor 5 (*CCR5*) gene and the E/E genotype of the K469E SNP in the intercellular adhesion molecule 1 (*ICAM-1*) gene may confer population specific effects[80,81,93]. Both genes are plausible candidate genes in PSC. The CCR5 may be involved in the recruitment of intestinally activated lymphocytes *via* portal expression of CCR5 ligands (e.g. the macrophage inflammatory protein-1α and β), and ICAM-1 may play a similar role in recruiting leukocytes to an inflamed liver by interacting with the β2-integrin ligand. The negative findings in the replication series referred to in Table 1 state it unlikely that genetic variants of these receptors are of primary importance in the pathogenesis of PSC. The receptors may, however, still be involved in the disease process along with other CCRs and adhesion molecules [e.g. CCR9 and the mucosal addressin cell adhesion molecule 1 (MAdCAM-1)[94,95]].

GENETIC ASSOCIATIONS WITH CLINICAL SUBSETS OF PSC PATIENTS

The most prominent features of PSC along with the biliary changes are inflammatory bowel disease, cholangiocarcinoma and other autoimmune diseases (Figure 1).

The increased frequency of autoimmune diseases among patients with PSC is possibly due to the increased frequency of the AH8.1 among the patients[58,96]. Similarly, an increased frequency of IBD risk alleles among patients with PSC could contribute to the co-occurrence of these two phenotypes. Several IBD susceptibility genes have been identified during the last 6 years through the application of genome-wide linkage screens and subsequent fine-mapping approaches[26]. To determine if the high frequency of IBD among patients with PSC could be due to genetic risk factors shared with IBD in general, we recently genotyped key polymorphisms of known IBD susceptibility genes in a large cohort of Scandinavian PSC patients[97]. The following genes were studied: caspase activating recruitment domain 15 (*CARD15*), toll-like receptor 4 (*TLR-4*), caspase activating recruitment domain 4 (*CARD4*), solute carrier family 22, member 4 and 5 (*SLC22A4* and *SLC22A5*), Drosophila discs large homolog 5 (*DLG5*) and multidrug resistance gene 1 (*MDR1*)[26,98]. No significant PSC associations were detected for any of the investigated polymorphisms[97]. These negative findings add to notions that the IBD phenotype in PSC may be a "third" IBD phenotype[99], possibly distinct from UC and Crohn's disease not only in clinical presentation, but also with regard to genetic susceptibility.

It is of interest to know whether genetic associations detected in PSC may be of particular importance for the IBD phenotype among the PSC patients or patients with IBD in general. In a recent study of HLA alleles in

PSC and UC patients of the same ethnicity[100], the only parallel association detected was a protective effect of the DRB1*0404 allele, more pronounced among the PSC patients than among the patients with UC without liver disease. No association with any of the main PSC risk alleles (DRB1*0301, DRB1*1301 or DRB1*1501) was found among the regular UC patients. Interestingly, a non-significant trend towards a higher frequency of the DRB1*1501 allele was noted among the patients with PSC and concurrent IBD compared with PSC patients without IBD, and the possibility should be held open that this HLA haplotype may harbour genetic variants of particular importance for the IBD phenotype in PSC. A similar notion can be made with regard to the MMP3 5A allele association detected by Satsangi *et al*[79]. Although the replication study by Wiencke *et al*[78] failed to confirm an overall association with PSC susceptibility, a significant association was evident when PSC patients with UC were compared with UC patients without liver disease.

The study by Wiencke *et al*[78] also detected a possible association between cholangiocarcinoma and the MMP1 1G allele. Although the number of patients with cholangiocarcinoma in this series was too small for conclusive statistics to be performed (n = 15), the 100% occurrence of this allele among the cholangiocarcinoma patients warrants future replication attempts in other study populations. Recently, a highly significant association between polymorphisms in the *NKG2D* gene and cholangiocarcinoma in PSC was detected[71]. Previous studies have highlighted the importance of this activating NK cell receptor in protection against other cancer types[66]. Persistent exposure to effector molecules of inflammatory pathways (e.g. IL-6[101]), along with chronic cholestasis[102], is probably important for the malignant transformation of cholangiocytes. The study by Melum *et al*[33] points to the possible role of NK cell activity in protection against neoplastic cells. Polymorphisms of the *NKG2D* gene along with other parameters may also prove important in identifying PSC patients at a particular low risk of developing cholangiocarcinoma.

MODIFIER GENES IN PSC

There is an increasing interest in so-called "modifier genes" in complex diseases (as compared with "susceptibility genes"), initiated by the recognition of the influence of such genes on disease expression (e.g. severity) in monogenic disorders like cystic fibrosis and haemochromatosis[103-105]. Modifier genes may point to biochemical and physiological systems of relevance to prognosis and are therefore of great clinical interest. Although PSC should be considered a progressive condition culminating in death or liver transplantation in most cases[106], the clinical course for each individual patient varies considerably[107,108]. In terms of disease course, indicators of PSC severity (e.g. portal hypertension and need for liver transplantation) are more likely to represent a particular disease stage than to serve as valid measures of disease progression. The most precise strategy for performing enquiries on effects from genotypes on disease course in PSC is thus to compare absolute survival time (defined as time from diagnosis until death or liver transplantation) using Kaplan-Meyer analyses, or calculating the relative risk for death and/or liver transplantation from Cox regressions[109,110].

We have recently observed that genetic variants of the steroid and xenobiotic receptor (SXR) are associated with a more aggressive disease course in PSC[110]. The SXR is a ligand-dependent transcription factor known to mediate protection against bile acid-induced liver injury in cholestatic animal models[111,112]. In this perspective, our data may suggest that the activity of bile acid detoxification systems could be of importance for disease progression in PSC. Interestingly, the SXR ligand rifampicin has been used in the treatment of cholestatic pruritus[113], and it has also been shown that ursodeoxycholic acid is able to activate SXR in human hepatocytes[114]. However, the SXR may also influence inflammatory pathways *via* the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB)[115], as well as liver fibrogenesis and thus cirrhosis *via* direct effects on hepatic stellate cells and Kuppfer cells[116]. Further studies are needed to clarify the functional consequences of various polymorphisms of the *SXR* gene in patients with PSC.

The SXR variants associated with death or liver transplantation in our study were not associated with PSC susceptibility[110]. However, also for some of the disease-associated variants in the HLA complex, modifier effects have been observed. The first notion was made by Gow *et al*[117] who described an unusually aggressive disease progression in four patients carrying the DR4 allele. Later, Boberg *et al*[109] found that DR4 positive patients have an increased risk of cholangiocarcinoma, but do formally not experience an accelerated disease progression. In this study, an increased risk of death or liver transplantation was observed in patients heterozygous for the DR3-DQ2 haplotype. As long as the causative variants along the HLA haplotypes in question have not been identified, one can only hypothesize upon a biological explanation for these observations. Given the complexity of the HLA associations in PSC, it is even possible that other variants within this region may be important for disease progression than those primarily important for disease susceptibility. However, for the same reasons it has been difficult to pinpoint susceptibility genes in this region (strong LD, multiple genes of immunological relevance, *etc*.), such modifier genes may prove hard to identify conclusively.

FUTURE STUDIES AND CONCLUDING REMARKS

Although several important findings have been made during the past 25 years since the first genetic association study in PSC was performed[24], PSC remains an enigmatic disease and future studies are warranted. With an ever increasing availability of methods for efficient genotyping of polymorphisms[118], a critical limitation for such studies in PSC is the availability of well-characterised patient materials. Collaborative efforts will be necessary to achieve patient collections required for detecting the modest effects (Figure 2), as well as for replicating results of

uncertain validity[33]. Such collaborations are now being undertaken in other diseases[119], and have successfully aided in clarifying genetic associations found in PSC[37].

In terms of future research strategies, several proposals can be made. First, dissection of the widely replicated HLA-associated susceptibility to PSC should be considered a priority. Detailed maps of genetic markers in this region are now available[120]. It is anticipated that the systematic application of such marker maps in populations of an appropriate size may lead to the identification of true, disease causing variants in this difficult region[62].

Second, some biological pathways are pointed to by existing findings (e.g. the possible importance of bile acid homeostasis in influencing disease progression), and further candidate gene studies of critical components of these systems may identify additional risk factors. There is increasing awareness of the importance of interaction between polymorphisms in functionally related genes in complex diseases, i.e. epistasis[121,122]. In some cases, epistatic considerations have proven necessary for the detection of effects from genetic variation on a phenotype of interest[123,124]. These observations have implications for study design in future candidate gene studies in PSC. Polymorphisms not only in single genes, but in relevant panels of several genes encoding proteins with closely related functions, should be investigated.

Finally, two recent advances in the genetic research field now make genome-wide studies feasible also for case-control materials. First, the human haplotype map project (HAPMAP) was recently completed[125]. In the project, 3.9 million SNPs have been genotyped in families of three different ethnicities (at the time of writing). Results from the project enable researchers worldwide to efficiently select SNPs throughout the genome that are prone to cover genetic variation of interest to a project[126,127]. Second, although costs are high, genotyping technology now allows for the typing of 100 000's of SNPs simultaneously in the same DNA sample[118]. Emerging reports provide proof-of-concept for genome-wide case-control studies[128,129]. However, there are still statistical problems to be solved regarding the many tests performed and risk of false positive results[130]. As evident from Figure 2, only strong effects may be detectable, and prospects may not yet justify the costs. However, sooner or later genome-wide studies seem warranted, also in PSC. Possibly, PSC susceptibility genes will be identified that would otherwise never have been included in hypothesis-driven candidate gene studies of the type performed so far[131].

REFERENCES

1 **Schwartz si**, Dale WA. Primary sclerosing cholangitis; review and report of six cases. *AMA Arch Surg* 1958; **77**: 439-451

2 **Chapman RW**, Arborgh BA, Rhodes JM, Summerfield JA, Dick R, Scheuer PJ, Sherlock S. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. *Gut* 1980; **21**: 870-877

3 **Cullen SN**, Chapman RW. Review article: current management of primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2005; **21**: 933-948

4 **Olsson R**, Boberg KM, de Muckadell OS, Lindgren S, Hultcrantz R, Folvik G, Bell H, Gangsoy-Kristiansen M, Matre J, Rydning A, Wikman O, Danielsson A, Sandberg-Gertzen H, Ung KA, Eriksson A, Loof L, Prytz H, Marschall HU, Broome U. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. *Gastroenterology* 2005; **129**: 1464-1472

5 **Brandsaeter B**, Friman S, Broome U, Isoniemi H, Olausson M, Backman L, Hansen B, Schrumpf E, Oksanen A, Ericzon BG, Hockerstedt K, Makisalo H, Kirkegaard P, Bjoro K. Outcome following liver transplantation for primary sclerosing cholangitis in the Nordic countries. *Scand J Gastroenterol* 2003; **38**: 1176-1183

6 **Talwalkar JA**, Lindor KD. Primary sclerosing cholangitis. *Inflamm Bowel Dis* 2005; **11**: 62-72

7 **Boberg KM**, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand J Gastroenterol* 1998; **33**: 99-103

8 **Kingham JG**, Kochar N, Gravenor MB. Incidence, clinical patterns, and outcomes of primary sclerosing cholangitis in South Wales, United Kingdom. *Gastroenterology* 2004; **126**: 1929-1930

9 **Bambha K**, Kim WR, Talwalkar J, Torgerson H, Benson JT, Therneau TM, Loftus EV Jr, Yawn BP, Dickson ER, Melton LJ 3rd. Incidence, clinical spectrum, and outcomes of primary sclerosing cholangitis in a United States community. *Gastroenterology* 2003; **125**: 1364-1369

10 **Schrumpf E**, Boberg KM. Epidemiology of primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2001; **15**: 553-562

11 **Broome U**, Olsson R, Loof L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzen H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610-615

12 **Okolicsanyi L**, Fabris L, Viaggi S, Carulli N, Podda M, Ricci G. Primary sclerosing cholangitis: clinical presentation, natural history and prognostic variables: an Italian multicentre study. The Italian PSC Study Group. *Eur J Gastroenterol Hepatol* 1996; **8**: 685-691

13 **Escorsell A**, Pares A, Rodes J, Solis-Herruzo JA, Miras M, de la Morena E. Epidemiology of primary sclerosing cholangitis in Spain. Spanish Association for the Study of the Liver. *J Hepatol* 1994; **21**: 787-791

14 **Takikawa H**, Takamori Y, Tanaka A, Kurihara H, Nakanuma Y. Analysis of 388 cases of primary sclerosing cholangitis in Japan; Presence of a subgroup without pancreatic involvement in older patients. *Hepatol Res* 2004; **29**: 153-159

15 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429

16 **Fausa O**, Schrumpf E, Elgjo K. Relationship of inflammatory bowel disease and primary sclerosing cholangitis. *Semin Liver Dis* 1991; **11**: 31-39

17 **Rasmussen HH**, Fallingborg JF, Mortensen PB, Vyberg M, Tage-Jensen U, Rasmussen SN. Hepatobiliary dysfunction and primary sclerosing cholangitis in patients with Crohn's disease. *Scand J Gastroenterol* 1997; **32**: 604-610

18 **Saarinen S**, Olerup O, Broome U. Increased frequency of autoimmune diseases in patients with primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 3195-3199

19 **Bergquist A**, Ekbom A, Olsson R, Kornfeldt D, Loof L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzen H, Almer S, Granath F, Broome U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327

20 **Schrumpf E**, Abdelnoor M, Fausa O, Elgjo K, Jenssen E, Kolmannskog F. Risk factors in primary sclerosing cholangitis. *J Hepatol* 1994; **21**: 1061-1066

21 **Boberg KM**, Jebsen P, Clausen OP, Foss A, Aabakken L, Schrumpf E. Diagnostic benefit of biliary brush cytology in cholangiocarcinoma in primary sclerosing cholangitis. *J Hepatol* 2006; **45**: 568-574

22 **Lazaridis KN**, Gores GJ. Primary sclerosing cholangitis and cholangiocarcinoma. *Semin Liver Dis* 2006; **26**: 42-51

23 **Loftus EV Jr**, Sandborn WJ, Tremaine WJ, Mahoney DW, Zinsmeister AR, Offord KP, Melton LJ 3rd. Primary sclerosing

cholangitis is associated with nonsmoking: a case-control study. *Gastroenterology* 1996; **110**: 1496-1502
24 **Schrumpf E**, Fausa O, Forre O, Dobloug JH, Ritland S, Thorsby E. HLA antigens and immunoregulatory T cells in ulcerative colitis associated with hepatobiliary disease. *Scand J Gastroenterol* 1982; **17**: 187-191
25 **Donaldson PT**, Norris S. Immunogenetics in PSC. *Best Pract Res Clin Gastroenterol* 2001; **15**: 611-627
26 **Gaya DR**, Russell RK, Nimmo ER, Satsangi J. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet* 2006; **367**: 1271-1284
27 **Rioux JD**, Abbas AK. Paths to understanding the genetic basis of autoimmune disease. *Nature* 2005; **435**: 584-589
28 **Glazier AM**, Nadeau JH, Aitman TJ. Finding genes that underlie complex traits. *Science* 2002; **298**: 2345-2349
29 **Bergquist A**, Lindberg G, Saarinen S, Broome U. Increased prevalence of primary sclerosing cholangitis among first-degree relatives. *J Hepatol* 2005; **42**: 252-256
30 **Mathew CG**, Lewis CM. Genetics of inflammatory bowel disease: progress and prospects. *Hum Mol Genet* 2004; **13** Spec No 1: R161-R168
31 **Strachan T**, Read A. Human Molecular Genetics, 2nd edn. Oxford: BIOS Scientific Publishers Ltd, 1999
32 **Venter JC**, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. *Science* 2001; **291**: 1304-1351
33 **Melum E**, Karlsen TH, Broome U, Thorsby E, Schrumpf E, Boberg KM, Lie BA. The 32-base pair deletion of the chemokine receptor 5 gene (CCR5-Delta32) is not associated with primary sclerosing cholangitis in 363 Scandinavian patients. *Tissue Antigens* 2006; **68**: 78-81
34 **Zondervan KT**, Cardon LR. The complex interplay among factors that influence allelic association. *Nat Rev Genet* 2004; **5**: 89-100
35 **Terwilliger JD**, Weiss KM. Linkage disequilibrium mapping of complex disease: fantasy or reality? *Curr Opin Biotechnol* 1998; **9**: 578-594
36 **Weiss KM**, Terwilliger JD. How many diseases does it take to map a gene with SNPs? *Nat Genet* 2000; **26**: 151-157
37 **Spurkland A**, Saarinen S, Boberg KM, Mitchell S, Broome U, Caballeria L, Ciusani E, Chapman R, Ercilla G, Fausa O, Knutsen I, Pares A, Rosina F, Olerup O, Thorsby E, Schrumpf E. HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. *Tissue Antigens* 1999; **53**: 459-469
38 **Colhoun HM**, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003; **361**: 865-872
39 **Trikalinos TA**, Ntzani EE, Contopoulos-Ioannidis DG, Ioannidis JP. Establishment of genetic associations for complex diseases is independent of early study findings. *Eur J Hum Genet* 2004; **12**: 762-769
40 **Risch N**, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; **273**: 1516-1517
41 **Cardon LR**, Bell JI. Association study designs for complex diseases. *Nat Rev Genet* 2001; **2**: 91-99
42 **Hill AB**. The Environment and Disease: Association or Causation? *Proc R Soc Med* 1965; **58**: 295-300
43 **Horton R**, Wilming L, Rand V, Lovering RC, Bruford EA, Khodiyar VK, Lush MJ, Povey S, Talbot CC Jr, Wright MW, Wain HM, Trowsdale J, Ziegler A, Beck S. Gene map of the extended human MHC. *Nat Rev Genet* 2004; **5**: 889-899
44 **Dawkins R**, Leelayuwat C, Gaudieri S, Tay G, Hui J, Cattley S, Martinez P, Kulski J. Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. *Immunol Rev* 1999; **167**: 275-304
45 **Marsh SGE**, Parham P, Barber LD. The HLA factsbook. London, San Diego: Academic Press Inc., 1999
46 **Parham P**. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol* 2005; **5**: 201-214
47 **Dorak MT**, Shao W, Machulla HK, Lobashevsky ES, Tang J, Park MH, Kaslow RA. Conserved extended haplotypes of the major histocompatibility complex: further characterization. *Genes Immun* 2006; **7**: 450-467
48 **Ahmad T**, Neville M, Marshall SE, Armuzzi A, Mulcahy-Hawes K, Crawshaw J, Sato H, Ling KL, Barnardo M, Goldthorpe S, Walton R, Bunce M, Jewell DP, Welsh KI. Haplotype-specific linkage disequilibrium patterns define the genetic topography of the human MHC. *Hum Mol Genet* 2003; **12**: 647-656
49 **Blomhoff A**, Olsson M, Johansson S, Akselsen HE, Pociot F, Nerup J, Kockum I, Cambon-Thomsen A, Thorsby E, Undlien DE, Lie BA. Linkage disequilibrium and haplotype blocks in the MHC vary in an HLA haplotype specific manner assessed mainly by DRB1*03 and DRB1*04 haplotypes. *Genes Immun* 2006; **7**: 130-140
50 **Chapman RW**, Varghese Z, Gaul R, Patel G, Kokinon N, Sherlock S. Association of primary sclerosing cholangitis with HLA-B8. *Gut* 1983; **24**: 38-41
51 **Donaldson PT**, Farrant JM, Wilkinson ML, Hayllar K, Portmann BC, Williams R. Dual association of HLA DR2 and DR3 with primary sclerosing cholangitis. *Hepatology* 1991; **13**: 129-133
52 **Moloney MM**, Thomson LJ, Strettell MJ, Williams R, Donaldson PT. Human leukocyte antigen-C genes and susceptibility to primary sclerosing cholangitis. *Hepatology* 1998; **28**: 660-662
53 **Norris S**, Kondeatis E, Collins R, Satsangi J, Clare M, Chapman R, Stephens H, Harrison P, Vaughan R, Donaldson P. Mapping MHC-encoded susceptibility and resistance in primary

sclerosing cholangitis: the role of MICA polymorphism. *Gastroenterology* 2001; **120**: 1475-1482
54 **Wiencke K**, Spurkland A, Schrumpf E, Boberg KM. Primary sclerosing cholangitis is associated to an extended B8-DR3 haplotype including particular MICA and MICB alleles. *Hepatology* 2001; **34**: 625-630
55 **Mitchell SA**, Grove J, Spurkland A, Boberg KM, Fleming KA, Day CP, Schrumpf E, Chapman RW. Association of the tumour necrosis factor alpha -308 but not the interleukin 10 -627 promoter polymorphism with genetic susceptibility to primary sclerosing cholangitis. *Gut* 2001; **49**: 288-294
56 **Neri TM**, Cavestro GM, Seghini P, Zanelli PF, Zanetti A, Savi M, Podda M, Zuin M, Colombo M, Floreani A, Rosina F, Bianchi Porro G, Strazzabosco M, Okolicsanyi L. Novel association of HLA-haplotypes with primary sclerosing cholangitis (PSC) in a southern European population. *Dig Liver Dis* 2003; **35**: 571-576
57 **Candore G**, Lio D, Colonna Romano G, Caruso C. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: effect of multiple gene interactions. *Autoimmun Rev* 2002; **1**: 29-35
58 **Price P**, Witt C, Allcock R, Sayer D, Garlepp M, Kok CC, French M, Mallal S, Christiansen F. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. *Immunol Rev* 1999; **167**: 257-274
59 **Donaldson PT**, Norris S. Evaluation of the role of MHC class II alleles, haplotypes and selected amino acid sequences in primary sclerosing cholangitis. *Autoimmunity* 2002; **35**: 555-564
60 **Wiencke K**, Karlsen TH, Boberg KM, Thorsby E, Schrumpf E, Lie BA, Spurkland A. Primary sclerosing cholangitis is associated with extended HLA-DR3 and HLA-DR6 haplotypes. *Tissue Antigens* 2007; **69**: 161-169
61 **Sollid LM**, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J Exp Med* 1989; **169**: 345-350
62 **Valentonyte R**, Hampe J, Huse K, Rosenstiel P, Albrecht M, Stenzel A, Nagy M, Gaede KI, Franke A, Haesler R, Koch A, Lengauer T, Seegert D, Reiling N, Ehlers S, Schwinger E, Platzer M, Krawczak M, Muller-Quernheim J, Schurmann M, Schreiber S. Sarcoidosis is associated with a truncating splice site mutation in BTNL2. *Nat Genet* 2005; **37**: 357-364
63 **Farrant JM**, Doherty DG, Donaldson PT, Vaughan RW, Hayllar KM, Welsh KI, Eddleston AL, Williams R. Amino acid substitutions at position 38 of the DR beta polypeptide confer susceptibility to and protection from primary sclerosing cholangitis. *Hepatology* 1992; **16**: 390-395
64 **Prochazka EJ**, Terasaki PI, Park MS, Goldstein LI, Busuttil RW. Association of primary sclerosing cholangitis with HLA-DRw52a. *N Engl J Med* 1990; **322**: 1842-1844
65 **Inability to attribute susceptibility to primary sclerosing cholangitis to specific amino acid positions of the HLA-DRw52a allele**. *N Engl J Med* 1991; **325**: 1251-1252
66 **Hayashi T**, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. *Cancer Res* 2006; **66**: 563-570
67 **Karlsen TH**, Boberg KM, Olsson M, Sun JY, Senitzer D, Bergquist A, Schrumpf E, Thorsby E, Lie BA. Particular genetic variants of ligands for natural killer cell receptors may contribute to the HLA associated risk of primary sclerosing cholangitis. *J Hepatol* 2007; **46**: 899-906
68 **Nelson GW**, Martin MP, Gladman D, Wade J, Trowsdale J, Carrington M. Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. *J Immunol* 2004; **173**: 4273-4276
69 **Gambelunghe G**, Ghaderi M, Tortoioli C, Falorni A, Santeusanio F, Brunetti P, Sanjeevi CB, Falorni A. Two distinct MICA gene markers discriminate major autoimmune diabetes types. *J Clin Endocrinol Metab* 2001; **86**: 3754-3760
70 **Stastny P**. Introduction: MICA/MICB in innate immunity, adaptive immunity, autoimmunity, cancer, and in the immune response to transplants. *Hum Immunol* 2006; **67**: 141-144
71 **Melum E**, Karlsen TH, Boberg KM, Bergquist A, Thorsby E, Schrumpf E, Lie BA. Genetic variation in the receptor-ligand pair NKG2D-MICA is strongly associated with development of cholangiocarcinoma in patients with primary sclerosing cholangitis. *J Hepatol* 2007; **46**: S49
72 **Hashimoto E**, Lindor KD, Homburger HA, Dickson ER, Czaja AJ, Wiesner RH, Ludwig J. Immunohistochemical characterization of hepatic lymphocytes in primary biliary cirrhosis in comparison with primary sclerosing cholangitis and autoimmune chronic active hepatitis. *Mayo Clin Proc* 1993; **68**: 1049-1055
73 **Hata K**, Van Thiel DH, Herberman RB, Whiteside TL. Phenotypic and functional characteristics of lymphocytes isolated from liver biopsy specimens from patients with active liver disease. *Hepatology* 1992; **15**: 816-823
74 **Silvain C**, Zeevi A, Saidman S, Duquesnoy RJ, Van Thiel DH. Phenotypic and functional characteristics of colonic lymphocytes isolated from patients with primary sclerosing cholangitis and inflammatory bowel disease. *Hepatogastroenterology* 1995; **42**: 250-258
75 **Abdalian R**, Heathcote EJ. Sclerosing cholangitis: a focus on secondary causes. *Hepatology* 2006; **44**: 1063-1074
76 **Stokkers PC**, van Aken BE, Basoski N, Reitsma PH, Tytgat GN, van Deventer SJ. Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease. *Gut* 1998; **43**: 33-39
77 **Donaldson PT**, Norris S, Constantini PK, Bernal W, Harrison P, Williams R. The interleukin-1 and interleukin-10 gene polymorphisms in primary sclerosing cholangitis: no associations with disease susceptibility/resistance. *J Hepatol* 2000; **32**: 882-886
78 **Wiencke K**, Louka AS, Spurkland A, Vatn M, Schrumpf E, Boberg KM. Association of matrix metalloproteinase-1 and -3 promoter polymorphisms with clinical subsets of Norwegian primary sclerosing cholangitis patients. *J Hepatol* 2004; **41**: 209-214
79 **Satsangi J**, Chapman RW, Haldar N, Donaldson P, Mitchell S, Simmons J, Norris S, Marshall SE, Bell JI, Jewell DP, Welsh KI. A functional polymorphism of the stromelysin gene (MMP-3) influences susceptibility to primary sclerosing cholangitis. *Gastroenterology* 2001; **121**: 124-130
80 **Eri R**, Jonsson JR, Pandeya N, Purdie DM, Clouston AD, Martin N, Duffy D, Powell EE, Fawcett J, Florin TH, Radford-Smith GL. CCR5-Delta32 mutation is strongly associated with primary sclerosing cholangitis. *Genes Immun* 2004; **5**: 444-450
81 **Yang X**, Cullen SN, Li JH, Chapman RW, Jewell DP. Susceptibility to primary sclerosing cholangitis is associated with polymorphisms of intercellular adhesion molecule-1. *J Hepatol* 2004; **40**: 375-379
82 **Bowlus CL**, Karlsen TH, Broome U, Thorsby E, Vatn M, Schrumpf E, Lie BA, Boberg KM. Analysis of MAdCAM-1 and ICAM-1 polymorphisms in 365 Scandinavian patients with primary sclerosing cholangitis. *J Hepatol* 2006; **45**: 704-710
83 **Girodon E**, Sternberg D, Chazouilleres O, Cazeneuve C, Huot D, Calmus Y, Poupon R, Goossens M, Housset C. Cystic fibrosis transmembrane conductance regulator (CFTR) gene defects in patients with primary sclerosing cholangitis. *J Hepatol* 2002; **37**: 192-197
84 **Sheth S**, Shea JC, Bishop MD, Chopra S, Regan MM, Malmberg E, Walker C, Ricci R, Tsui LC, Durie PR, Zielenski J, Freedman SD. Increased prevalence of CFTR mutations and variants and decreased chloride secretion in primary sclerosing cholangitis. *Hum Genet* 2003; **113**: 286-292
85 **Gallegos-Orozco JF**, E Yurk C, Wang N, Rakela J, Charlton MR, Cutting GR, Balan V. Lack of association of common cystic fibrosis transmembrane conductance regulator gene mutations with primary sclerosing cholangitis. *Am J Gastroenterol* 2005; **100**: 874-878
86 **Pauli-Magnus C**, Kerb R, Fattinger K, Lang T, Anwald B, Kullak-Ublick GA, Beuers U, Meier PJ. BSEP and MDR3

haplotype structure in healthy Caucasians, primary biliary cirrhosis and primary sclerosing cholangitis. *Hepatology* 2004; **39**: 779-791

87 **Rosmorduc O**, Hermelin B, Boelle PY, Poupon RE, Poupon R, Chazouilleres O. ABCB4 gene mutations and primary sclerosing cholangitis. *Gastroenterology* 2004; **126**: 1220-1222; author reply 1222-1223

88 **Vogel A**, Liermann H, Harms A, Strassburg CP, Manns MP, Obermayer-Straub P. Autoimmune regulator AIRE: evidence for genetic differences between autoimmune hepatitis and hepatitis as part of the autoimmune polyglandular syndrome type 1. *Hepatology* 2001; **33**: 1047-1052

89 **Stokkers PC**, de Heer K, Leegwater AC, Reitsma PH, Tytgat GN, van Deventer SJ. Inflammatory bowel disease and the genes for the natural resistance-associated macrophage protein-1 and the interferon-gamma receptor 1. *Int J Colorectal Dis* 1999; **14**: 13-17

90 **Wiencke K**, Boberg KM, Donaldson P, Harbo H, Ling V, Schrumpf E, Spurkland A. No major effect of the CD28/CTLA4/ICOS gene region on susceptibility to primary sclerosing cholangitis. *Scand J Gastroenterol* 2006; **41**: 586-591

91 **Karlsen TH**, Flåm S, Schrumpf E, Broomé U, Thorsby E, Vatn M, Boberg KM, Lie BA. Polymorphisms of the forkhead box P3 gene on chromosome X in primary sclerosing cholangitis and ulcerative colitis. *Genes Immun* 2005; **6**: 56 (abstract)

92 **Popov Y**, Patsenker E, Fickert P, Trauner M, Schuppan D. Mdr2 (Abcb4)-/- mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. *J Hepatol* 2005; **43**: 1045-1054

93 **Henckaerts L**, Fevery J, Van Steenbergen W, Verslype C, Yap P, Nevens F, Roskams T, Libbrecht L, Rutgeerts P, Vermeire S. CC-type chemokine receptor 5-Delta32 mutation protects against primary sclerosing cholangitis. *Inflamm Bowel Dis* 2006; **12**: 272-277

94 **Adams DH**, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. *Nat Rev Immunol* 2006; **6**: 244-251

95 **O'Mahony CA**, Vierling JM. Etiopathogenesis of primary sclerosing cholangitis. *Semin Liver Dis* 2006; **26**: 3-21

96 **Cullen S**, Chapman R. Primary sclerosing cholangitis. *Autoimmun Rev* 2003; **2**: 305-312

97 **Karlsen TH**, Hampe J, Wiencke K, Schrumpf E, Thorsby E, Lie BA, Broome U, Schreiber S, Boberg KM. Genetic polymorphisms associated with inflammatory bowel disease do not confer risk for primary sclerosing cholangitis. *Am J Gastroenterol* 2007; **102**: 115-121

98 **Ho GT**, Soranzo N, Nimmo ER, Tenesa A, Goldstein DB, Satsangi J. ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype tagging approach. *Hum Mol Genet* 2006; **15**: 797-805

99 **Loftus EV Jr**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96

100 **Karlsen TH**, Boberg KM, Vatn M, Bergquist A, Hampe J, Schrumpf E, Thorsby E, Schreiber S, Lie BA. Different HLA class II associations in ulcerative colitis patients with and without primary sclerosing cholangitis. *Genes Immun* 2007; **8**: 275-278

101 **Kobayashi S**, Werneburg NW, Bronk SF, Kaufmann SH, Gores GJ. Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells. *Gastroenterology* 2005; **128**: 2054-2065

102 **Komichi D**, Tazuma S, Nishioka T, Hyogo H, Chayama K. Glycochenodeoxycholate plays a carcinogenic role in immortalized mouse cholangiocytes via oxidative DNA damage. *Free Radic Biol Med* 2005; **39**: 1418-1427

103 **Drumm ML**, Konstan MW, Schluchter MD, Handler A, Pace R, Zou F, Zariwala M, Fargo D, Xu A, Dunn JM, Darrah RJ, Dorfman R, Sandford AJ, Corey M, Zielenski J, Durie P, Goddard K, Yankaskas JR, Wright FA, Knowles MR. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med* 2005; **353**: 1443-1453

104 **Jacolot S**, Le Gac G, Scotet V, Quere I, Mura C, Ferec C. HAMP as a modifier gene that increases the phenotypic expression of the HFE pC282Y homozygous genotype. *Blood* 2004; **103**: 2835-2840

105 **Le Gac G**, Scotet V, Ka C, Gourlaouen I, Bryckaert L, Jacolot S, Mura C, Ferec C. The recently identified type 2A juvenile haemochromatosis gene (HJV), a second candidate modifier of the C282Y homozygous phenotype. *Hum Mol Genet* 2004; **13**: 1913-1918

106 **Porayko MK**, LaRusso NF, Wiesner RH. Primary sclerosing cholangitis: a progressive disease? *Semin Liver Dis* 1991; **11**: 18-25

107 **Schrumpf E**, Fausa O, Elgjo K, Kolmannskog F. Hepatobiliary complications of inflammatory bowel disease. *Semin Liver Dis* 1988; **8**: 201-209

108 **Balasubramaniam K**, Wiesner RH, LaRusso NF. Primary sclerosing cholangitis with normal serum alkaline phosphatase activity. *Gastroenterology* 1988; **95**: 1395-1398

109 **Boberg KM**, Spurkland A, Rocca G, Egeland T, Saarinen S, Mitchell S, Broome U, Chapman R, Olerup O, Pares A, Rosina F, Schrumpf E. The HLA-DR3,DQ2 heterozygous genotype is associated with an accelerated progression of primary sclerosing cholangitis. *Scand J Gastroenterol* 2001; **36**: 886-890

110 **Karlsen TH**, Lie BA, Frey Froslie K, Thorsby E, Broome U, Schrumpf E, Boberg KM. Polymorphisms in the steroid and xenobiotic receptor gene influence survival in primary sclerosing cholangitis. *Gastroenterology* 2006; **131**: 781-787

111 **Xie W**, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, Evans RM. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci USA* 2001; **98**: 3375-3380

112 **Stedman CA**, Liddle C, Coulter SA, Sonoda J, Alvarez JG, Moore DD, Evans RM, Downes M. Nuclear receptors constitutive androstane receptor and pregnane X receptor ameliorate cholestatic liver injury. *Proc Natl Acad Sci USA* 2005; **102**: 2063-2068

113 **Khurana S**, Singh P. Rifampin is safe for treatment of pruritus due to chronic cholestasis: a meta-analysis of prospective randomized-controlled trials. *Liver Int* 2006; **26**: 943-948

114 **Schuetz EG**, Strom S, Yasuda K, Lecureur V, Assem M, Brimer C, Lamba J, Kim RB, Ramachandran V, Komoroski BJ, Venkataramanan R, Cai H, Sinal CJ, Gonzalez FJ, Schuetz JD. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. *J Biol Chem* 2001; **276**: 39411-39418

115 **Zhou C**, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, Lin M, Mallick S, Forman BM, Thummel KE, Blumberg B. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest* 2006; **116**: 2280-2289

116 **Wright MC**. The impact of pregnane X receptor activation on liver fibrosis. *Biochem Soc Trans* 2006; **34**: 1119-1123

117 **Gow PJ**, Fleming KA, Chapman RW. Primary sclerosing cholangitis associated with rheumatoid arthritis and HLA DR4: is the association a marker of patients with progressive liver disease? *J Hepatol* 2001; **34**: 631-635

118 **Hirschhorn JN**, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005; **6**: 95-108

119 **Rich SS**, Concannon P, Erlich H, Julier C, Morahan G, Nerup J, Pociot F, Todd JA. The Type 1 Diabetes Genetics Consortium. *Ann N Y Acad Sci* 2006; **1079**: 1-8

120 **de Bakker PI**, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J, Ke X, Monsuur AJ, Whittaker P, Delgado M, Morrison J, Richardson A, Walsh EC, Gao X, Galver L, Hart J, Hafler DA, Pericak-Vance M, Todd JA, Daly MJ, Trowsdale J, Wijmenga C, Vyse TJ, Beck S, Murray SS, Carrington M, Gregory S, Deloukas P, Rioux JD. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 2006; **38**: 1166-1172

121 **Gregersen JW**, Kranc KR, Ke X, Svendsen P, Madsen LS, Thomsen AR, Cardon LR, Bell JI, Fugger L. Functional

epistasis on a common MHC haplotype associated with multiple sclerosis. *Nature* 2006; **443**: 574-577
122 **Cordell HJ**. Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum Mol Genet* 2002; **11**: 2463-2468
123 **Khakoo SI**, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, Cheng J, Goedert JJ, Vlahov D, Hilgartner M, Cox S, Little AM, Alexander GJ, Cramp ME, O'Brien SJ, Rosenberg WM, Thomas DL, Carrington M. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 2004; **305**: 872-874
124 **Martin MP**, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, Trowsdale J, Wilson M, O'Brien SJ, Carrington M. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet* 2002; **31**: 429-434
125 **A haplotype map of the human genome**. *Nature* 2005; **437**: 1299-1320
126 **Conrad DF**, Jakobsson M, Coop G, Wen X, Wall JD, Rosenberg NA, Pritchard JK. A worldwide survey of haplotype variation and linkage disequilibrium in the human genome. *Nat Genet* 2006; **38**: 1251-1260
127 **de Bakker PI**, Burtt NP, Graham RR, Guiducci C, Yelensky R, Drake JA, Bersaglieri T, Penney KL, Butler J, Young S, Onofrio RC, Lyon HN, Stram DO, Haiman CA, Freedman ML, Zhu X, Cooper R, Groop L, Kolonel LN, Henderson BE, Daly MJ, Hirschhorn JN, Altshuler D. Transferability of tag SNPs in genetic association studies in multiple populations. *Nat Genet* 2006; **38**: 1298-1303
128 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463
129 **Yang Z**, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, Li X, Chien J, Dewan A, Harmon J, Bernstein PS, Shridhar V, Zabriskie NA, Hoh J, Howes K, Zhang K. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science* 2006; **314**: 992-993
130 **Wang WY**, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet* 2005; **6**: 109-118
131 **Hampe J**, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Gunther S, Prescott NJ, Onnie CM, Hasler R, Sipos B, Folsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207-211
132 **Jeffreys AJ**, Ritchie A, Neumann R. High resolution analysis of haplotype diversity and meiotic crossover in the human TAP2 recombination hotspot. *Hum Mol Genet* 2000; **9**: 725-733

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CLINICAL RESEARCH

Ribavirin and IFN-α combination therapy induces CD4+ T-cell proliferation and Th1 cytokine secretion in patients with chronic hepatitis B

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Abstract

AIM: To investigate the anti-viral mechanism of combination therapy of interferon (IFN)-α and ribavirin in patients with chronic hepatitis B.

METHODS: Twenty patients were assigned to receive either IFN-α plus ribavirin (group A, *n* = 14) or no treatment as a control (group B, *n* = 6). Patients were analyzed for T-cell proliferative responses specific for hepatitis B virus (HBV)-antigen and cytokine production by peripheral blood mononuclear cells (PBMCs).

RESULTS: Combination therapy induced HBV-antigen specific CD4+ T-cell proliferative responses in four patients (28.6%). Production of high levels of HBV-specific IFN-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-12 by PBMCs was found in five patients (35.7%), who showed significantly lower HBV DNA levels in serum at 12 mo after treatment ended (P = 0.038) and at 24 mo of follow-up (P = 0.004) than those without high levels of cytokine production.

CONCLUSION: HBV-antigen specific CD4+ T cells may directly control HBV replication and secretion of anti-viral T helper 1 (Th1) cytokines by PBMCs during combination therapy of chronic hepatitis B with ribavirin and IFN-α.

Key words: Hepatitis B; Interferon-alpha; Ribavirin; CD4+ T cells; Th1

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INTRODUCTION

More than 400 million people worldwide have chronic hepatitis B virus (HBV) infections[1]. Chronically infected patients with active liver disease have a high risk of developing cirrhosis and hepatocellular carcinoma[2]. However, therapeutic options against HBV still present a major clinical challenge. The goal of treatment is HBV DNA suppression, normalization of alanine aminotransferase (ALT) levels and reduction in liver necroinflammation. Currently available therapies against HBV are mainly interferon (IFN)-α and nucleoside analogs, which are well tolerated and induce a decrease in serum HBV DNA levels and normalization of serum ALT levels. However, the efficacy of IFN-α[3,4] or nucleoside analogs for treatment of hepatitis B varies in different clinical situations[5-12]. IFN-α shows seroconversion from hepatitis B e antigen (HBeAg) to antibody to HBeAg (anti-HBe), concomitant with HBV DNA negativity in just one-third of patients treated, and is both costly and induces adverse effects[3]. It has been well established that IFN-α has potent antiviral activity against DNA and RNA viruses, and that it also acts as an immunomodulatory agent[13]. Some reports have suggested that ribavirin shows antiviral and immune effects against various infections[14], including hepatitis B and C. Both drugs have the capacity to modulate systemic as well as virus-specific T-cell responses, along with the potential to shift the profile of cytokine secretion[15,16].

Recent reports have suggested that combination therapy with IFN-α plus ribavirin for chronic hepatitis B significantly reduces viremia[17,18] and induces lasting CD4+ T-cell proliferation and Th1 cytokine release at the site of infection, which may lead to sustained HBV eradication[18]. These preliminary data in anti-HBe-positive patients refractory to IFN-α treatment appear to be promising[18]. Thus, in the present study, we investigated the mechanism involved in the control of HBV replication, utilizing combination therapy with ribavirin and IFN-α.

MATERIALS AND METHODS

Patients

Twenty patients with chronic hepatitis B (14 men and 6 women; mean age 42 years), positive for both anti-HBe and HBV DNA in the serum, and who had failed previous IFN-α treatment were enrolled in this prospective trial. None had human immunodeficiency virus, hepatitis

C virus, or hepatitis D virus infections, hepatocellular carcinoma, or had received nucleoside analogs. Six healthy controls were also analyzed (mean age 38 years). Table 1 shows patient characteristics at enrollment. This study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by institutional ethics committee. Informed consent was obtained from all patients prior to inclusion.

Therapeutic and analytic schedule

The patients were divided into two groups: group A (n = 14) for combination therapy with IFN-α plus ribavirin, and group B (n = 6) for untreated controls. Patients in group A received 5 million U IFN-α2b three times a week for 12 mo, plus ribavirin (1000 mg/d) orally for 12 mo[17]. Patients were followed for 12 mo after the end of treatment. Blood chemistry, blood cell counts, and HBV DNA were measured at the beginning of treatment, and then every 1-2 mo during treatment and follow-up periods. HBeAg and anti-HBe were measured at the beginning of treatment, and then at 6-mo intervals. Blood samples for immunological analysis were collected before therapy and at 3, 6, 9, 12, 18 and 24 mo after the commencement of therapy. Serum HBV DNA was quantified, using transcription-mediated amplification and a hybridization assay. The concentration of HBV DNA in the samples was expressed as the logarithm of genome equivalents per milliliter (LGE/mL). Biochemical and hematologic parameters were measured by standard methods. All patients completed treatment and follow-up.

Cell preparation

The methods of cell preparation used here were nearly identical to those of Ren *et al*[19] in their report focusing on therapeutic vaccination against chronic hepatitis B. Peripheral blood mononuclear cells (PBMCs) from 20 patients were separated from heparinized blood by density-gradient centrifugation with lymphoprep (Nycomed Pharma AS, Oslo, Norway). B cells were removed from PBMCs by negative depletion, by incubating the cells with mouse anti-$CD19^+$ antibodies coated on magnetic beads (Danal, Olso, Norway). CD4+ or CD8+ T cells were then removed from the resultant T cells in the same manner, using mouse anti-human CD4+ or CD8+ antibodies coated on magnetic beads (Danal), respectively. CD4+ cells were also blocked with CD4+ antibodies (Caltag Laboratories, Burlingame, CA, USA). The purity of the T-cell subpopulation was monitored by immunolabeling with anti-$CD3^+$ antibodies (Becton Dickinson, San Jose, CA, USA). Flow cytometry revealed a > 95% purified T-cell subpopulation. PBMC or lymphocyte subsets were resuspended with 2 mmol/L l-glutamine, 10 mmol/L HEPES, 100 kU/L penicillin, 100 mg/L streptomycin, and 50 mL/L human AB serum (complete medium).

Proliferation assay

The proliferation assay used in this study was nearly identical to that of Ren *et al*[19]. T cells (1.5×10^6 cell/L in 0.2 mL complete medium) were cultured in triplicate wells of 96-well round-bottom microplates with medium alone, were stimulated with 10 mg/L phytohemagglutinin (PHA: Sigma) and 3 mg/L HBV antigens: HBsAg protein, synthetic entire preS1, HBeAg, and hepatitis B core antigen (HbcAg) (Virostat, Portland, ME, USA). After 4 d of culture at 37℃ in an atmosphere of 50 mL/L CO_2 in air, the cells were labeled for 18 h with 37 kBq of [^{3}H]-thymidine (Amersham, Little Chalfont, UK). DNA-incorporated radioactivity was measured by scintillation counting. Data were expressed as the stimulation index (SI), calculated as the ratio of the mean cpm of triplicate cultures obtained in the presence of antigen to cpm obtained without antigen. SI > 3 was considered significant. The proliferative responses were not tested with PBMCs from patients in control group B.

Table 1 Clinical characteristics of patients at enrollment

Patient No.	Age (yr)	Gender	ALT (nkat/L)	HBV DNA (10^3 LGE/L)	Type of response (end of treatment)
Group A					
1	48	M	89	6.8	Responder
2	51	M	158	7.3	Responder
3	44	M	86	8.3	Responder
4	41	M	464	8.0	Responder
5	39	M	122	4.8	Responder
6	67	M	142	8.1	Non-responder
7	52	M	140	8.3	Non-responder
8	26	M	69	4.8	Non-responder
9	52	M	46	7.6	Non-responder
10	33	M	39	5.4	Non-responder
11	24	F	458	8.7	Non-responder
12	27	F	51	8.5	Non-responder
13	52	F	80	7.4	Non-responder
14	37	F	57	6.8	Non-responder
Group B					
15	42	F	43	7.2	No treatment
16	33	F	19	8.1	No treatment
17	51	M	138	3.9	No treatment
18	31	M	57	7.1	No treatment
19	29	M	79	7.1	No treatment
20	44	M	46	7.2	No treatment

P > 0.05, Group A *vs* Group B.

Cytokine assay

The cytokine assay used in this study was nearly identical to that of Ren *et al*[19]. PBMCs (1.5×10^6 cell/L in 0.2 mL complete medium) were cultured in triplicate wells of 96-well round-bottom microplates with medium alone, and stimulated with 10 mg/L PHA or with 3 mg/L HBsAg, HBeAg, HBcAg or preS1. After 3 d of culture at 37℃ in an atmosphere of 50 mL/L CO_2 in air, culture supernatants were collected. Concentrations of IFN-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-4, IL-10 and IL-12 p70 were determined using commercial ELISA kits (Genzyme, Cambridge, MA, USA). Production levels after antigen stimulation were expressed as the ratio of the mean cytokine concentration of triplicate cultures obtained in the presence of antigen to that obtained without antigen.

Statistical analysis

Results are expressed as mean ± SD. Differences in proportions were tested by the χ^2 test. Mean quantitative values were compared using the Mann-Whitney U test. All reported P values were two-tailed and $P \leqslant 0.05$ was considered significant.

Table 2 SI of T cells against HBV antigens from combination therapy patients

Patient No.	12 mo					24 mo				
	PHA	HBsAg	preS1	HBeAg	HBcAg	PHA	HBsAg	preS1	HBeAg	HBcAg
1	38.1[1]	3.5[1]	3.2[1]	3.9[1]	4.2[1]	8.2[1]	4.1[1]	3.5[1]	4.1[1]	4.8[1]
2	20.3[1]	3.4[1]	4.0[1]	4.1[1]	4.4[1]	7.7[1]	3.2[1]	3.0	4.4[1]	4.7[1]
3	19.5[1]	3.2[1]	3.9[1]	4.8[1]	4.1[1]	4.9[1]	3.5[1]	3.7[1]	4.7[1]	5.1[1]
4	32.3[1]	4.3[1]	7.8[1]	7.2[1]	7.9[1]	9.6[1]	4.0[1]	6.9[1]	8.1[1]	8.8[1]
5	15.5[1]	1.9	2.3	2.0	1.7	8.4[1]	3.5[1]	3.5[1]	4.3[1]	4.4[1]
6	12.8[1]	0.4	1.2	1.5	1.4	4.9[1]	1.1	1.3	1.5	1.2
7	50.2[1]	2.1	1.9	2.0	2.0	38.8[1]	1.7	1.8	1.6	1.6
8	4.9[1]	1.8	2.1	1.7	1.9	NT	NT	NT	NT	NT
9	66.3[1]	2.0	1.5	1.8	1.6	12.0[1]	1.5	1.2	1.5	1.3
10	56.6[1]	2.3	2.3	2.0	2.1	18.3[1]	2.0	2.1	1.8	1.9
11	23.8[1]	1.1	1.0	1.1	1.2	8.7[1]	1.0	0.8	1.2	1.3
12	19.2[1]	1.4	1.2	1.4	1.3	4.6[1]	1.1	1.5	1.1	1.2
13	42.0[1]	1.8	1.5	1.9	1.7	6.9[1]	1.5	1.3	1.4	1.5
14	21.8[1]	2.2	2.4	2.2	2.6	4.9[1]	2.0	2.3	1.9	2.0

[1]SI > 3 correspond to significant proliferative responses. NT, not tested; PHA (10 mg/L); HBV antigen (3 mg/L).

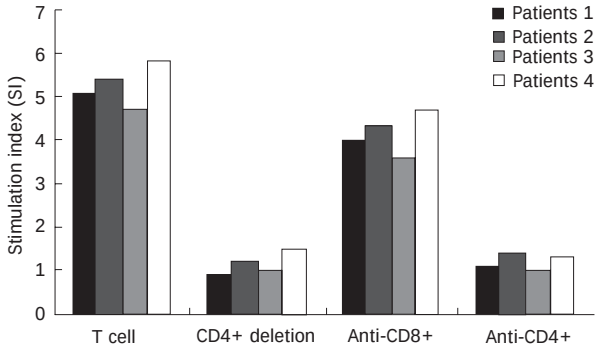

Figure 1 Abrogation of antigen-specific T-cell proliferative responses from patients 1-4 at 12 mo ([b]$P < 0.01$ *vs* T cell and Anti CD8+).

RESULTS

Clinical outcome

HBV DNA levels decreased, with a significant reduction at 9 mo and thereafter, as compared to those at baseline, in the combination therapy patients. Serum HBV DNA levels were also significantly lower in the combination therapy patients than in the controls at 12 and 24 mo. At 12 mo, four patients in group A (28.6%) (patients 1-4) had undetectable HBV DNA levels, and also showed sustained normalization of ALT; they were thus considered sustained responders, as previously reported[17,18]. The remaining 10 patients at 12 mo had detectable HBV DNA and elevated ALT levels.

Induction of T-cell proliferation response to HBV antigens

Proliferative responses of T cells during combination therapy and the follow-up period are summarized in Table 2. Four patients (1-4) showed significant proliferative responses at 12 mo, and these responses were sustained until 24 mo (the end of follow-up). These proliferative responses were always specific to both HBV antigens. Thus, this combination therapy was found to have induced proliferative T cell responses specific to the antigen contained in these four patients (28.6%). Patient 5 also showed a strong proliferative response at 24 mo. However, whether the combination therapy induced this response is unclear because it occurred at 12 mo after completion of therapy.

T-cell proliferative responses were also examined for patients 1-4 after incubation with anti-CD4+ antibodies or removing CD4+ or CD8+ cells. Depletion of CD8+ cells did not clearly inhibit the proliferative responses, while depletion of CD4+ cells or blocking with anti-CD4+ antibodies completely abrogated the proliferative responses of the patients (Figure 1).

HBV-specific cytokine production in PBMCs

HBV-specific cytokine production levels of PBMCs are shown in Figure 2. Cytokine production showed a Th1-like pattern characterized by secretion of IFN-γ, TNF-α, and IL-12 in the absence of IL-4 and IL-10 in five patients (1-5, defined as responders). Patients 1-4 exhibited remarkable increases in IFN-α, TNF-α, and IL-12 production at 3, 6 or 9 mo, as compared to patient 5 who showed mild, but not significant, proliferative responses (Table 2). These responses were sustained until the end of the observation period. Production of Th1 (IFN-γ, TNF-α, and IL-12) and Th2 (IL-4 and IL-10) cytokines did not increase, or remained unchanged in the patients who received other combination therapy (patients 6-14, defined as non-responders).

The mean serum HBV DNA level was lower in responders than in non-responders at 12 mo (4.3 ± 1.1 *vs* 6.0 ± 1.1, $P = 0.038$) and 24 mo (3.8 ± 0 *vs* 5.7 ± 1.2, $P = 0.004$). It is noteworthy that HBV DNA fell to under the detection limit at 24 mo in all responders. The decrease in serum HBV DNA level was almost coincident with the increase in IFN-γ production by HBV antigen-specific T cells, but was not preceded by any increase in serum ALT levels in four responders (patients 2-5).

DISCUSSION

In the present study, a significant decrease was found in serum HBV DNA levels at 9 mo and thereafter, along with significantly lower levels of HBV DNA in the combination therapy patients than in the controls at 12 and 24 mo. IFN-α plus ribavirin therapy appeared to inhibit

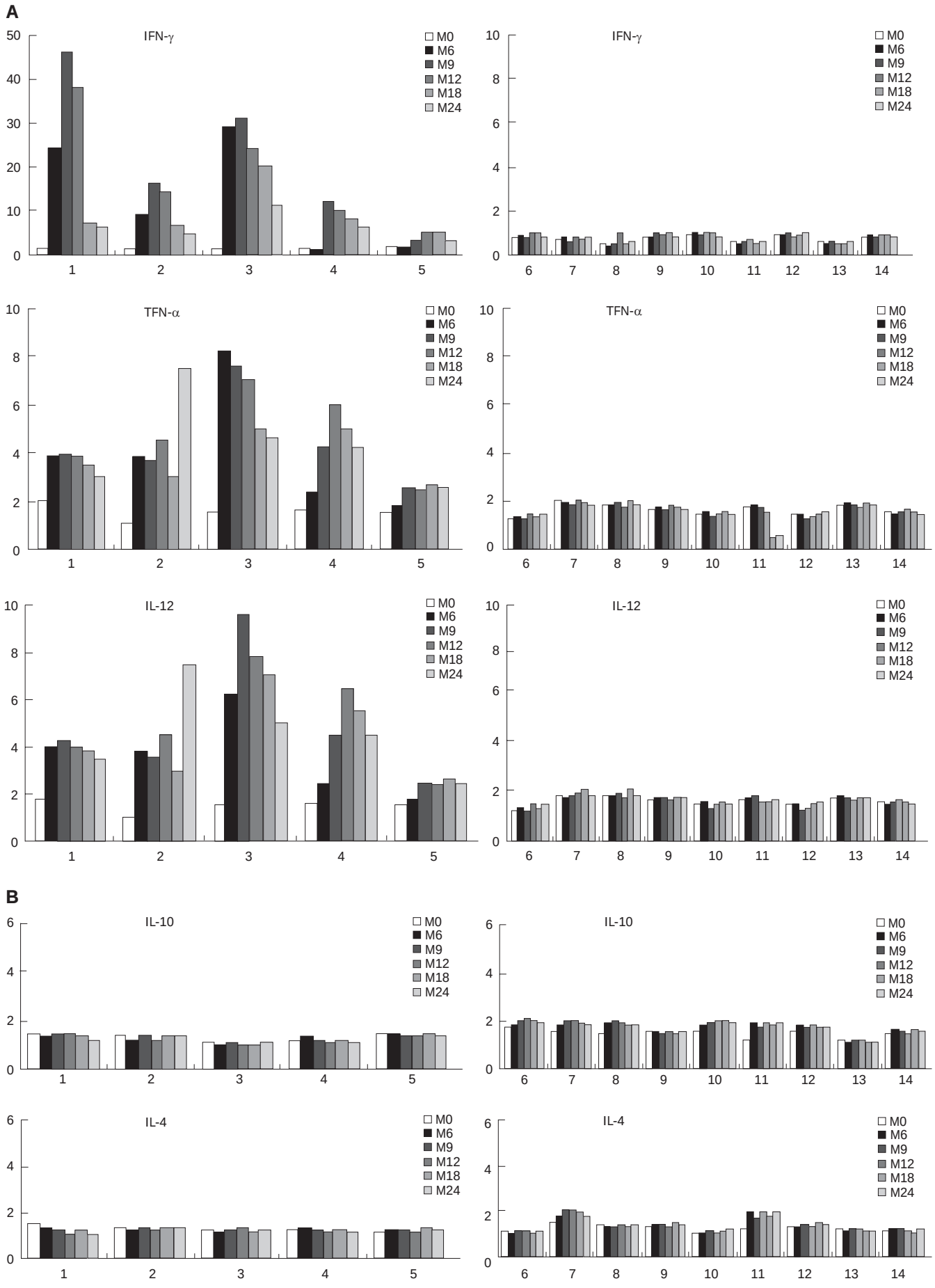

Figure 2 Cytokine production levels in PBMCs in combination therapy patients. The vertical axis represents the ratio of the mean cytokine concentration of triplicate cultures obtained in the presence of antigen to that obtained without antigen. The numbers in the horizontal lines represent the patients. **A:** Th1 cytokine production in PBMCs in combination therapy patients; **B:** Th2 cytokine production in PBMCs in combination therapy patients.

HBV replication in some patients, since serum HBV DNA levels were significantly lower at 12 and 24 mo in responders who showed HBV-antigen-specific IFN-γ, TNF-α and IL-12 production *in vitro* in PBMCs that was augmented in responders. The production of HBV-antigen-specific Th1 (IFN-γ, TNF-α, and IL-12) and Th2 (IL-4 and IL-10) cytokines did not increase, or remained unchanged in non-responders. Cytokine production showed a Th1-like pattern, as well as induction of PBMCs, and was consistent with the results of Rico *et al*[18].

CD4+ T cells are necessary for the maintenance of the effector functions of CD8+ T cells during chronic viral infection[20]. Activated CD8+ cytotoxic T cells can kill virus-infected cells by utilizing both perforin-dependent and Fas-mediated cytotoxic mechanisms[21]. CD8+ T cells can also secrete anti-viral cytokines such as IFN-γ and TNF-α[22]. The HBV-antigen-specific T-cell reactivity observed in our study may be relevant for the outcome of the infection, because it may be crucial to provide help to CD8+ cytotoxic T-cell responses to lyse and clear HBV-infected cells[23-27]. Nevertheless, eradication of HBV may be accomplished by other cells non-cytolytically by transcription and replication of HBV[28,29]. Based on our results, it is not possible to establish the mechanism contributing to HBV clearance (in this study we did not investigate CD8+ cytotoxic T cells in the cytotoxic assay). However, the decrease in serum HBV DNA levels was almost coincident with the increase of IFN-γ production by antigen-specific CD4+ T cells and was not preceded by an increase in serum ALT levels (as represented by patient 2, data not shown). These results suggest that cytotoxic T cells are unlikely to contribute to the control of HBV replication in combination therapy with ribavirin and IFN-α; results that are supported by those of Rico *et al*[18]. CD4+ T cells appear to directly participate in the anti-viral response (by producing anti-viral cytokines) rather than indirectly (by helping cytotoxic T cells) in this combination therapy of ribavirin and IFN-α. A role for Th1 cells in controlling viral infection is supported by experiments showing that they can clear influenza[30,31] and vaccinia virus[32] infections in a cytotoxic-T-lymphocyte-independent manner. Further, a direct, cytokine-dependent anti-viral role for CD4+ T cells, which produce Th1 cytokines (Th1 cells), has been shown in HBV transgenic mice[33,34]. These reports support our concept that the increased production of anti-viral cytokines by PBMCs plays a crucial role in the control of HBV replication in combination therapy with IFN-α and ribavirin. This combination therapy for chronic hepatitis B not only significantly reduced viremia levels but also induced lasting CD4+ T-cell proliferation and Th1 cytokine release at the site of infection, which may have led to sustained HBV eradication, as suggested by Rico *et al*[18]. Further studies will be needed to ascertain whether the anti-viral mechanism of combination therapy is by a route different from the one normally employed.

In conclusion, the present study indicated that combination therapy with ribavirin and IFN-α for anti-HBe-positive patients significantly reduces viremia, and induces CD4+ T-cell proliferation and Th1 cytokine secretion in patients with chronic hepatitis B.

COMMENTS

Background

Some recent reports have suggested that ribavirin shows antiviral and immune effects against various infectious diseases, including hepatitis B and C. It is suggested that combination therapy with IFN-α plus ribavirin for chronic hepatitis B significantly reduces viremia; however, the mechanisms involved remain unclear.

Research frontiers

Previous studies have suggested that combination therapy with IFN-α plus ribavirin for chronic hepatitis B significantly reduces viremia; however, the mechanism is unclear. In the present study, we investigated the anti-viral mechanism of combination therapy with IFN-α and ribavirin against chronic hepatitis B by analyzing T-cell proliferative responses in patients and determining Th1 cytokine levels.

Innovations and breakthroughs

In this study, we analyzed HBV-specific CD4+ T-cell proliferative responses and determined Th1 cytokine levels in PBMCs. Our results indicated that combination therapy of patients with chronic hepatitis B with ribavirin and IFN-α significantly reduced viremia, and induced CD4+ T-cell proliferation and Th1 cytokine secretion.

Applications

This study indicates that combination therapy with ribavirin and IFN-α for chronic hepatitis B significantly reduces viremia; thus, this combination may represent an alternative treatment option to achieve sustained eradication of HBV in patients with chronic hepatitis B refractory to IFN-α treatment.

Peer review

This is an interesting report of combination therapy with IFN-α plus ribavirin against chronic hepatitis B. The results suggest that HBV-antigen-specific CD4+ T cells may directly control HBV replication and secretion of anti-viral Th1 cytokines by PBMCs, utilizing combination therapy with ribavirin and IFN-α against chronic hepatitis B.

REFERENCES

1 **Lai CL**, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. *Lancet* 2003; **362**: 2089-2094
2 **Chisari FV**, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29-60
3 **Wong DK**, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993; **119**: 312-323
4 **Sokal E**. Drug treatment of pediatric chronic hepatitis B. *Pediatr Drugs* 2002; **4**: 361-369
5 **Lai CL**, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61-68
6 **Lai CL**, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011-1020
7 **Dienstag JL**, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, Condreay LD, Woessner M, Rubin M, Brown NA. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; **341**: 1256-1263
8 **Chang TT**, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, Pastore G, Bacon BR, Poynard T, Joshi S, Klesczewski KS, Thiry A, Rose RE, Colonno RJ, Hindes RG. A dose-ranging study of the efficacy and tolerability of entecavir in Lamivudine-refractory chronic hepatitis B patients. *Gastroenterology* 2005; **129**: 1198-1209
9 **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
10 **Hadziyannis SJ**, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart CL. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* 2003; **348**: 800-807
11 **Hadziyannis SJ**, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart CL. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005; **352**: 2673-2681
12 **Sherman M**, Yurdaydin C, Sollano J, Silva M, Liaw YF, Cianciara J, Boron-Kaczmarska A, Martin P, Goodman Z, Colonno R, Cross A, Denisky G, Kreter B, Hindes R. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006; **130**: 2039-2049
13 **Tilg H**. New insights into the mechanisms of interferon alfa: an immunoregulatory and anti-inflammatory cytokine. *Gastroenterology* 1997; **112**: 1017-1021
14 **Fernandez H**, Banks G, Smith R. Ribavirin: a clinical overview. *Eur J Epidemiol* 1986; **2**: 1-14
15 **Hultgren C**, Milich DR, Weiland O, Sallberg M. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. *J Gen Virol* 1998; **79** (Pt 10): 2381-2391
16 **Martin J**, Navas S, Quiroga JA, Pardo M, Carreno V. Effects of the ribavirin-interferon alpha combination on cultured peripheral blood mononuclear cells from chronic hepatitis C patients. *Cytokine* 1998; **10**: 635-644
17 **Cotonat T**, Quiroga JA, Lopez-Alcorocho JM, Clouet R, Pardo M, Manzarbeitia F, Carreno V. Pilot study of combination therapy with ribavirin and interferon alfa for the retreatment of chronic hepatitis B e antibody-positive patients. *Hepatology* 2000; **31**: 502-506
18 **Rico MA**, Quiroga JA, Subira D, Castanon S, Esteban JM, Pardo M, Carreno V. Hepatitis B virus-specific T-cell proliferation and cytokine secretion in chronic hepatitis B e antibody-positive patients treated with ribavirin and interferon alpha. *Hepatology* 2001; **33**: 295-300
19 **Ren F**, Hino K, Yamaguchi Y, Funatsuki K, Hayashi A, Ishiko H, Furutani M, Yamasaki T, Korenaga K, Yamashita S, Konishi T, Okita K. Cytokine-dependent anti-viral role of CD4-positive T cells in therapeutic vaccination against chronic hepatitis B viral infection. *J Med Virol* 2003; **71**: 376-384
20 **Matloubian M**, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. *J Virol* 1994; **68**: 8056-8063
21 **Smyth MJ**, Trapani JA. The relative role of lymphocyte granule exocytosis versus death receptor-mediated cytotoxicity in viral pathophysiology. *J Virol* 1998; **72**: 1-9
22 **Guidotti LG**, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 1996; **4**: 25-36
23 **Lohr HF**, Weber W, Schlaak J, Goergen B, Meyer zum Buschenfelde KH, Gerken G. Proliferative response of CD4+ T cells and hepatitis B virus clearance in chronic hepatitis with or without hepatitis B e-minus hepatitis B virus mutants. *Hepatology* 1995; **22**: 61-68
24 **Lohr HF**, Krug S, Herr W, Weyer S, Schlaak J, Wolfel T, Gerken G, Meyer zum Buschenfelde KH. Quantitative and functional analysis of core-specific T-helper cell and CTL activities in acute and chronic hepatitis B. *Liver* 1998; **18**: 405-413
25 **Jung MC**, Hartmann B, Gerlach JT, Diepolder H, Gruber R, Schraut W, Gruner N, Zachoval R, Hoffmann R, Santantonio T, Wachtler M, Pape GR. Virus-specific lymphokine production differs quantitatively but not qualitatively in acute and chronic hepatitis B infection. *Virology* 1999; **261**: 165-172
26 **Marinos G**, Naoumov NV, Williams R. Impact of complete inhibition of viral replication on the cellular immune response in chronic hepatitis B virus infection. *Hepatology* 1996; **24**: 991-995
27 **Wild J**, Grusby MJ, Schirmbeck R, Reimann J. Priming MHC-I-restricted cytotoxic T lymphocyte responses to exogenous hepatitis B surface antigen is CD4+ T cell dependent. *J Immunol* 1999; **163**: 1880-1887
28 **Guidotti LG**, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science* 1999; **284**: 825-829
29 **Protzer U**, Nassal M, Chiang PW, Kirschfink M, Schaller H. Interferon gene transfer by a hepatitis B virus vector efficiently suppresses wild-type virus infection. *Proc Natl Acad Sci USA* 1999; **96**: 10818-10823
30 **Eichelberger M**, Allan W, Zijlstra M, Jaenisch R, Doherty PC. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted CD8+ T cells. *J Exp Med* 1991; **174**: 875-880
31 **Scherle PA**, Palladino G, Gerhard W. Mice can recover from pulmonary influenza virus infection in the absence of class I-restricted cytotoxic T cells. *J Immunol* 1992; **148**: 212-217
32 **Spriggs MK**, Koller BH, Sato T, Morrissey PJ, Fanslow WC, Smithies O, Voice RF, Widmer MB, Maliszewski CR. Beta 2-microglobulin-, CD8+ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses. *Proc Natl Acad Sci USA* 1992; **89**: 6070-6074
33 **Franco A**, Guidotti LG, Hobbs MV, Pasquetto V, Chisari FV. Pathogenetic effector function of CD4-positive T helper 1 cells in hepatitis B virus transgenic mice. *J Immunol* 1997; **159**: 2001-2008
34 **Guidotti LG**, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; **19**: 65-91

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CLINICAL RESEARCH

Influence of a nucleotide oligomerization domain 1 (*NOD1*) polymorphism and *NOD2* mutant alleles on Crohn's disease phenotype

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Abstract

AIM: To examine genetic variation of nucleotide oligomerization domain 1 (*NOD1*) and *NOD2*, their respective influences on Crohn's disease phenotype and gene-gene interactions.

METHODS: (*ND1+32656*1*) *NOD1* polymorphism and *SNP8*, *SNP12* and *SNP13* of *NOD2* were analyzed in 97 patients and 50 controls. *NOD2* variants were determined by reaction restriction fragment length polymorphism analysis. *NOD1* genotyping and *NOD2* variant confirmation were performed by specific amplification and sequencing.

RESULTS: The distribution of *NOD1* polymorphism in patients was different from controls (P = 0.045) and not altered by existence of *NOD2* mutations. In this cohort, 30.92% patients and 6% controls carried at least one *NOD2* variant (P < 0.001) with R702W being the most frequent variant. Presence of at least one *NOD2* mutation was inversely associated with colon involvement (9.09% with colon *vs* 36.4% with ileal or ileocolonic involvement, P = 0.04) and indicative of risk of penetrating disease (52.63% with penetrating *vs* 25.64% with non-penetrating or stricturing behavior, P = 0.02). L1007finsC and double *NOD2* mutation conferred the highest risk for severity of disease (26.3% with penetrating disease *vs* 3.8% with non-penetrating or stricturing behavior presented L1007finsC, P = 0.01 and 21.0% with penetrating disease *vs* 2.5% with non-penentrating or stricturing behavior carried double *NOD2* mutation, P = 0.007). Exclusion of patients with *NOD2* mutations from phenotype/*NOD1*-genotype analysis revealed higher prevalence of *1*1 genotype in groups of younger age at onset and colonic location.

CONCLUSION: This study suggests population differences in the inheritance of risk *NOD1* polymorphism and *NOD2* mutations. Although no interaction between *NOD1*-*NOD2* was noticed, a relationship between disease location and Nod-like receptor molecules was established.

Key words: Crohn's disease; Nucleotide oligomerization domain 1; Nucleotide oligomerization domain 2

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. Although the etiopathogenesis of this disease remains poorly understood, both genetic and environmental factors have been suggested to predispose to CD. Various disease phenotypes, including age at diagnosis, sex, family history, location of disease, response to medical therapies and behavior of the disease may be genetically determined.

Experimental and observational data suggest that intestinal inflammation arises from abnormal immune reactivity to bacterial flora in the intestine of individuals who are genetically predisposed[1]. The analysis of the molecules that participate in the response of commensal organisms revealed that gastric and intestinal cells are

largely deficient in TLR signaling and must rely on alternative systems, such as Nod-like receptors (NLRs) for the detection of pathogens. The mammalian NLR family is composed of more than 20 members that share a modular domain organization of a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide-binding site domain and a N-terminal protein-protein-interaction domain composed of a CARD (caspase activation and recruitment domain), pyrin domain or Bir domain[2].

The first NLRs reported to have a direct function as intracellular pattern recognition molecules were Nucleotide oligomerization domain 1 (*NOD1*) (*CARD4*) and *NOD2* (*CARD15*); both proteins detect distinct substructures from bacterial peptidoglycan. *NOD1* detects a unique tripeptide motif found in Gram-negative bacterial peptidoglycan and also in specific Gram-positive bacteria such as *Listeria* and *Bacillus* spp[3]. *NOD2* detects muramyl dipeptide, the largest molecular motif common to Gram-negative and Gram-positive bacteria[4]. It is expressed in intestinal epithelial cells, with high expression in Paneth cells in the small intestine, intestinal myofibroblasts, granulocytes, endothelial, and monocyte-derived cells[5,6].

Identification of *NOD2* as the first susceptibility gene for CD was a breakthrough in understanding inflammatory bowel disease (IBD) pathogenesis. *NOD2* gene is located at the CD susceptibility locus (*IBD1*) on chromosome 16q12[7,8] and it has more than 60 sequence variants. Although, disease-associated *NOD2* mutations linked to Blau syndrome and early onset of sarcoidosis have been found in the region encoding the nucleotide-binding site domain[9,10], the three common genetic mutations linked to CD are mapped within or adjacent to the LRR region of *NOD2* (leading to protein changes at R702W, G908R, L1007finsC)[7,8]. These mutations are associated with an altered NF-κB activation and the linkage is particularly strong with ileal and ileocolonic CD[11,12]. *NOD2* variants are associated with early surgery due to stenosis, postsurgical recurrence, familial CD[13] and stricturing and penetrating forms of CD[14].

CD association with *NOD2* has been widely replicated. However, investigations into the inheritance of the three risk alleles in *NOD2* associated with susceptibility to CD have demonstrated a remarkable heterogeneity across ethnicities and populations with regional variation across Europe[15,16].

The discovery of *NOD2*-related innate immune defects in certain CD cases has led to speculation about defects in other pattern recognition receptors and downstream signaling molecules. The gene encoding *NOD1* (*CARD4*) is located within the chromosome 7p14 IBD locus, a region that contains an IBD susceptibility locus in British families[17]. An association between a complex insertion/deletion polymorphism (*ND1+32656*1*) in *NOD1* and susceptibility to IBD has been described. Particularly, this polymorphism has been associated to age at diagnosis and to the presence of IBD extraintestinal manifestations[18]. This *NOD1* polymorphism has also been associated to increased susceptibility to asthma[19,20]. In both diseases, the mutation has been found to be an insertion/deletion polymorphism in an intron of *NOD1*. Convincing replication of these findings is pending, since no evidence of association between *ND1+32656*1* and IBD was found in two recent well-powered data sets[21,22].

The present study examines the genetic variation in *NOD1* and *NOD2* and their respective influences on the CD phenotype (age at diagnosis, disease location and behavior) in a cohort of well-characterized CD patients. Since *NOD1* and *NOD2* share structure and functions, a potential interaction between *NOD1* and *NOD2* variants in CD phenotype was analyzed. After stratifying patients by their *NOD2* genotype, the distribution of *NOD1* polymorphism was determined and the contribution of each genotype was studied in regard to the disease phenotype.

MATERIALS AND METHODS

Patients

Ninety-seven CD patients attending the IBD outpatient clinic of Hospital Sant Pau (Barcelona, Spain) were prospectively included in the study. Fifty healthy controls matched for age, sex and geography were also evaluated. CD diagnoses were based on clinical, radiologic, endoscopic and pathologic bases. Patients with CD were classified according to Montreal classification for age at onset, disease location and behavior[23]. All patients and healthy controls gave informed consent and the study was approved by the local ethics committee.

Genotyping

Analysis of *NOD2* variants was performed as previously described, using genomic DNA extracted from blood samples by Qiagen kit (Qiagen, Heiden, Germany). A panel of 3 single nucleotide polymorphisms (*SNP8*, *12* and *13*) was detected by a polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis (PCR-RFLP)[7]. Each *NOD2* variant was initially amplified by PCR using specific primers (Table 1). The PCR products were subsequently analyzed by restriction enzyme cleavage and gel electrophoresis. For assay of the SNP8, the PCR product (185 bp) was digested with MspI, resulting in the following fragments: 20, 35, 54 and 76 bp in R702 homozygous; 20, 35 and 130 bp in 702W homozygous and 20, 35, 54, 76, and 130 bp in heterozygous. For assay of the SNP12, the PCR product (163 bp) was digested with HhaI, resulting in the following fragments: 163 bp in G908 homozygous; 27 and 136 bp in 908R homozygous and 27, 136 and 163 in heterozygous. In order to detect the SNP13, the PCR product (151 bp) was digested with ApaI, resulting in the following fragments: 151 bp for Leu1007 homozygous; 20 and 131 bp in 1007Pro homozygous and 20, 131 and 151 bp in heterozygous.

Genotyping of *NOD1* (*ND1+32656*) polymorphism and confirmation of the three *NOD2* mutations were performed by specific amplification with the primers described in Table 1 and the subsequent sequencing of the amplified products. Sequencing reaction was performed using ABI PRISM BigDye terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed by Genescan analysis on an ABI Prism 3100 Genetic Analyser according to the manufacturer's protocol (Applied Biosystem).

Table 1 Primers for *NOD2* and *NOD1* genotyping

	Forward	Reverse	Size (bp)
NOD2			
R702W	5′-AGATCACAGCAGCCTTCCTG-3′	5′-CACGCTCTTGGCCTCACC-3′	185
G908R	5′-CTCTTTTGGCCTTTTCAGATTCTG-3′	5′-CAGCTCCTCCCTCTTCACCT-3′	163
L1007finsC	5′-GGCAGAAGCCCTCCTGCAGGGCC-3′	5′-CCTCAAAATTCTGCCATTCC-3′	151
NOD1			
ND1+32656	5′-TGACTGTGTGTGACTCTCTCTGC-3′	5′-TGGTGAAAGCTCTCCACTATCTC-3′	250

Table 2 Genotype at *NOD2* polymorphisms in CD cases and healthy controls

Mutation	Group	Genotype count, *n* (%)			OR (95% CI)[1]	*P*[2]
		WT/WT	Heterozygous	Homozygous		
R702W	CD	75 (77.32)	21 (21.65)	1 (1.03)	7.04 (1.58-31.30)	0.004
	Controls	48 (96.00)	2 (4.00)	0		
G908R	CD	92 (94.85)	5 (5.15)	0		0.166
	Controls	50 (100)	0	0		
L1007finsC	CD	89 (91.75)	8 (8.25)	0	4.40 (0.53-36.25)	0.167
	Controls	49 (98.00)	1 (2.00)	0		

[1]ORs and probability values for disease status associated with carriage of at least 1 mutant allele (heterozygous, compound heterozygous and homozygous were grouped together). [2]*P*-values were calculated with the Fisher's Exact test when comparing controls and CD patients.

Statistical analysis

Genotype and allele frequencies of the patients and controls were compared by the χ^2 test or Fisher exact test in 2×2 contingency tables with at least 1 expected value < 5. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate relative risks. A two-tailed *P* value $\leqslant 0.05$ was considered significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 14.0 for Windows (SPSS Inc., Chicago, Ⅲ).

RESULTS

Frequencies of three NOD2 mutant alleles and one NOD1 polymorphism in CD patients and healthy controls

NOD2 gene mutations (R702W, G908R and L1007finsC) were determined in 97 CD patients and 50 healthy controls. Frequencies are summarized in Table 2. The distribution of genotypes at each mutation was significantly different in CD patients versus controls. R702W was the most frequent variant in CD and controls (21.65% and 4%, respectively, $P = 0.004$), and the only homozygous mutant patient in this cohort was found for this SNP8. Carriage of R702W was associated to the highest risk for CD in our cohort of patients (OR = 7.04; 95% CI: 1.58-31.30). Genotype frequency of the L1007finsC variant was lower than R702W, but showed a tendency to be higher in CD patients than in controls (8.25% *vs* 2%, $P = 0.167$; OD 4.40, 95% CI: 0.53-36.25). The *NOD2* variant with the lowest frequency in CD patients and in controls was G908R (5.15% *vs* 0%, $P = 0.166$). No homozygous *NOD2* mutant was found for L1007finsC or G908R. In this CD cohort, 30.92% of patients carried at least one variant of *NOD2* compared with 6% of healthy controls ($P < 0.001$) (Table 3), conferring a high risk for CD (OR 7.01; 95% CI: 2.02-24.30). Six CD patients but no controls carried two *NOD2* variant alleles.

NOD1 complex insertion/deletion polymorphism (*ND1+32656*) was examined in the same cohort of patients and controls (Table 4). Fifty-two percent of controls were *1*1, 34% were *1*2 and 14% were *2*2, whereas 59.79% of CD patients were *1*1, 37.11% were *1*2 and only 3.09% were *2*2. The distribution of *NOD1* genotype according to the *ND1+32656* polymorphism in CD patients and controls was statistically different ($P = 0.045$). Frequency of CD patients carrying *1 allele was 96.8% whereas in controls it was 86%, conferring a significant risk to develop the disease (OR 5.10; 95% CI: 1.25-20.68, $P = 0.032$).

Distribution of *NOD1* genotype according to WT or mutant *NOD2* was analyzed in CD patients to assess potential interactions between NOD1 and NOD2 (Table 4). Among those patients carrying at least one *NOD2* mutant allele, 60% of the patients were *1 *1, 36.66% were *1 *2 and 3.33% were *2 *2. Similarly, 59.70% of NOD2 WT/WT were *1 *1, 37.31% were *1 *2 and 2.98% were *2 *2. The presence of *NOD2* mutant alleles had therefore no influence on the *NOD1* polymorphism distribution ($P = 0.99$), suggesting no gene-gene interactions.

Clinical characteristics of CD patients according to the NOD2 genotype

CD patients were classified according to Montreal classification, with minor modifications as indicated in Table 5. The association of *NOD2* mutations to each CD phenotype was analyzed using each mutant genotype. The presence of at least one risk allele or the joint analysis of

Table 3 Distribution of *NOD2* mutations in CD patients and controls

NOD2 genotype	CD Patients, *n* (%)	Controls, *n* (%)	OR (95% CI)[1]	*P*[2]
At least one variant[3]	30 (30.92)	3 (6)	7.01 (2.02-24.30)	< 0.001
Heterozygous	24 (24.74)	3 (6)	5.61 (1.59-19.0)	0.003
Compound heterozygous[4]	5 (5.15)	0		
Homozygous	1 (1.03)	0		

[1]ORs and probability values for disease status associated with *NOD2* genotype. [2]*P*-values are calculated with the Fisher's Exact test when comparing controls and CD patients. [3]At least one variant was considered if any subject had at least one copy of the variant allele. [4]Compound heterozygous was defined as the presence of two different variants.

Table 4 Genotype frequencies of *ND1+32656* in CD patients and controls

Group	*NOD2*	*n*[1]	*NOD1* genotype, *n* (%)		
			*1*1	*1*2	*2*2
CD		97	58 (59.79)[2]	36 (37.11)	3 (3.09)
	WT/WT	67	40 (59.70)	25 (37.31)	2 (2.98)
	Mutant[3]	30	18 (60.00)	11 (36.66)	1 (3.33)
Controls		50	26 (52.00)	17 (34.00)	7 (14.00)
	WT/WT	47	23 (48.93)	17 (36.17)	7 (14.89)
	Mutant	3	3 (100)	0	0

CD: Crohn's disease; WT: Wildtype. [1]Number of CD patients or controls; [2]Results are expressed as number of CD patients (% of CD patients); [3]NOD2 mutant was considered any subject that inherited at least one copy of the variant allele.

compound heterozygous and homozygous for *NOD2* mutations were considered as a single independent variable.

A high proportion of patients in this cohort were diagnosed under the age of 40 (A1 + A2, *n* = 2 + 78), whereas 17 patients were diagnosed over 40 years (A3). All 6 patients with 2 mutant *NOD2* alleles were diagnosed before 40 years of age.

To study the association between genotype and disease location, one L1 + L4 patient was included in the L1 group (*n* = 34, 35.05%), and another L2 + L4 patient was included in the L2 group (*n* = 23, 23.71%). *NOD2* WT/WT patients were similarly distributed in L1, L2 and L3 groups: in 21.65% patients disease location was terminal ileum (L1), in 20.62%, it was colonic (L2), and in 26.80%, it was ileocolonic (L3). However, location of disease in *NOD2* mutant patients was not identical to *NOD2* WT/WT patients (*P* = 0.08). Despite the fact that R702W polymorphism was the most frequent in this cohort, only one patient with colon location carried this mutation (the patient had a double *NOD2* mutation). The presence of at least one mutant *NOD2* gene was inversely associated with exclusively colonic involvement (L2) (*P* = 0.04, OR 0.26; 95% CI: 0.07-0.96). When analyzing compound heterozygous and homozygous *NOD2* mutations, 2.06% of patients were L1, 1.03% of patients were L2 and 3.09% of patients were L3, indicating a comparable distribution.

Similarly to location, behavior groups in CD patients were simplified as follows: B1 group included 4 patients B1p (*n* = 37, 38%), B2 included 4 patients B2p (*n* = 41, 42%) and B3 included 4 patients B3p (*n* = 19, 19.5%). Distribution of *NOD2* mutations was different depending on disease behavior (*P* = 0.003). The presence of at least one mutant *NOD2* allele was indicative of risk of penetrating disease (B3) (*P* = 0.02, OR 3.22, 95% CI: 1.14-9.06) with the allele L1007finsC being indicative of the highest risk (*P* = 0.007, OR 8.92; 95% CI: 1.91-41.68). The frequency of double *NOD2* mutants was significantly higher in the B3 group than in the B2 and B1 groups (66% of the double *NOD2* mutants were B3, 33.3% were B2 and non-double *NOD2* mutants were B1). Presence of two *NOD2* mutant alleles was therefore indicative of risk for severity of disease (*P* = 0.01, OR 10.13; 95% CI: 1.70-60.40). The exam of the behavior through the course of disease showed an expected changing pattern[24]. There was a progressive reduction in the proportion of patients in the group B1 (51.6% patients with 5 years of disease, 34.9% patients with 6-9 years of disease and 21.7% patients with 10-15 years of disease). Inversely, there was a progressive increase in the proportion of patients in the groups of more complicated forms, B2 (34.5% patients with 5 years of disease, 44.2 patients with 6-9 years of disease and 52.2% patients with 10-15 years of disease) and B3 (13.8% patients with 5 years of disease, 20.9% patients with 6-9 years of disease and 26.1% patients with 10-15 years of disease). Selecting the group of patients with 6-9 years of disease (*n* = 43), the presence of at least one NOD2 mutation was indicative of risk of penetrating disease (the B3 group) (*P* = 0.046, OR = 5.55, 95% CI: 1.14-27.01) and 66.7% of the double *NOD2* mutants were included in the B3 group. Twelve patients with perianal disease (B1p, B2p, and B3p) were analyzed separately. Four of these patients presented one *NOD2* mutation, one was a compound heterozygous and the rest were *NOD2* WT/WT.

Clinical characteristics of CD patients according to the NOD1 genotype

Phenotype of CD patients was analyzed according to the *ND1+32656* polymorphism of *NOD1* gene. Distribution of *NOD1* genotype according to older age at diagnosis (A3 *P* = 0.64), location (L1 *P* = 0.28, L2 *P* = 0.56 and L3 *P* = 0.26) and behavior (B1 *P* = 0.55, B2 *P* = 0.99 and B3 *P* = 0.99) of the disease were not different from healthy controls. Similarly to a previous report, ND1+32656 genotype distribution in the group of early-onset CD (A1 + A2) was different from that observed in healthy controls. Only 2.5% of CD patients in the early-onset group had *2*2 genotype compared to 14% of healthy controls (*P* = 0.04).

Since *NOD2* mutations have a strong association with some CD clinical characteristics, and in particular with the ileal location, 30 CD patients that presented at least one *NOD2* mutation were excluded from the phenotype/genotype study to prevent any influence of *NOD2* (Table 6). When comparing the distribution of *NOD1*

Table 5 Clinical characteristics of CD patients according to *NOD2* genotype

Clinical features	*n*[1]	*NOD2* genotype, *n* (%)					*P*[2] OR (95% CI)
		WT/WT	R702W/WT	G908R/WT	L1007fsinsC/WT	Heter.compound & homozygous	
Age at diagnosis							
< 40 yr (A1 + A2)	80	54 (55.67)[3]	13 (13.40)	3 (3.09)	4 (4.12)	6 (6.18)	0.57 1.56 (0.46-5.27)
> 40 yr (A3)	17	13 (13.40)	3 (3.09)	0	1 (1.03)	0	
Location							
Ileal (L1)	34	21 (21.65)	8 (8.25)	1 (1.03)	2 (2.06)	2 (2.06)	0.26 1.67 (0.69-4.06)
Colonic (L2)	23	20 (20.62)	0	1 (1.03)	1 (1.03)	1 (1.03)	0.04 0.26 (0.07-0.96)
Ileocolonic (L3)	40	26 (26.80)	8 (8.25)	1 (1.03)	2 (2.06)	3 (3.09)	0.5 1.38 (0.57-3.29)
Behavior							
Non-stricturing, non-penetrating (B1)	37	24 (24.74)	8 (8.25)	3 (3.09)	2 (2.06)	0	0.5 1.37 (0.56-3.29)
Stricturing (B2)	41	34 (35.05)	5 (5.15)	0	0	2 (2.06)	0.01 0.29 (0.11-0.78)
Penetrating (B3)	19	9 (9.28)	3 (3.09)	0	3 (3.09)	4 (4.12)	0.02 3.22 (1.14-9.06)

CD: Crohn's disease; WT: Wildtype. [1]Number of CD patients in each subgroup; [2]*P*-values, odds ratios and confidence intervals refer to the comparison of presence versus absence of the at least one mutant *NOD2* allele; [3]Results are expressed as number of CD patients (% of CD patients).

polymorphism in each phenotype group with healthy controls, a higher prevalence of *1*1 was observed in the group A1 + A2 ($P = 0.04$). Interestingly, there was a clear tendency of the colonic group (L2) to have a higher frequency of *1*1 and lower frequency of *1*2 and *2*2 than controls, but the distribution of the *ND1+32656* polymorphism was not statistically different because the low number of cases decreased the power of the test. Distribution of this *NOD1* polymorphism in the other clinical subgroups of CD patients was comparable to healthy controls. Seven of the 12 patients with perianal disease (B1p + B2p + B3p) were *NOD2* WT/WT. In this subgroup of patients, *NOD1* polymorphism analysis showed that three of them were *1*1 and four were *1*2.

Table 6 Clinical characteristics of *NOD2* WT/WT CD patients according to *NOD1* genotype

Clinical features	*n*[1]	*NOD2* WT/WT		
		NOD1: *1*1	*1*2	*2*2
Age at diagnosis				
< 40 yr (A1 + A2)	54	62.96[2]	35.18	1.85
> 40 yr (A3)	13	46.15	46.15	7.69
Location				
Ileal (L1)	21	57.14	38.09	4.76
Colonic (L2)	20	70	25	5
Ileocolonic (L3)	26	53.84	46.15	0
Behavior				
Non-stricturing, non-penetrating (B1)	24	62.5	33.33	4.16
Stricturing (B2)	34	61.76	35.29	2.94
Penetrating (B3)	9	44.44	55.55	0
Controls	47	48.93	36.17	14.89

WT: Wildtype. [1]Number of CD patients or controls in each subgroup; [2]Values are expressed as the percent of patients in each clinical subgroup.

DISCUSSION

The frequency of *NOD2* mutant alleles associated to CD in our cohort of patients was within the European range, but deviated somewhat from populations of nearby geographic regions[25,26]. The frequency of R702W was one of the highest described in Caucasian populations, whereas the frequency of L1007finsC was lower than in other studies[27]. This observation is consistent with marked racial and regional differences described in the inheritance of the three risk *NOD2* alleles[16].

As expected, carriage of *NOD2* mutations conferred a high risk for developing CD, but this was neither necessary nor sufficient for CD development. The three *NOD2* mutations were not equally involved in CD susceptibility. The presence of R702W showed the strongest risk for CD in our cohort of patients. However, this mutation was not associated to CD in Galician, Finnish or Scottish populations[26]. The mutation with the strongest CD association in several familial and non-familial studies was L1007finsC[14], but this was not so in our cohort. Although the frequency of L1007finsC was noticeably elevated in CD patients, the absence of controls with this genotype precluded a statistical comparison.

We found an association between the polymorphism located at the intron IX-exon IX boundary of *NOD1* and susceptibility to CD in our cohort of patients. These results confirm a previous report associating this *NOD1* polymorphism with early IBD-onset and extraintestinal manifestations[18]. Although one recent study did not show a significant association with IBD[21], this *NOD1* non-coding polymorphism showed a strong association with asthma and the presence of elevated IgE levels in three independent panels of subjects[20]. Other *NOD1* polymorphisms in the coding sequence have been previously examined and showed no influence in CD susceptibility[28]. Mutations with phenotypic effects should be predominantly found at the coding sequence but complex disease susceptibility is often mediated through regulatory polymorphisms. In this case, *ND1+32656* may affect the binding of an unknown nuclear factor[20]. The involvement of *NOD1* gene is not surprising, since *NOD1*, similarly to *NOD2*, is involved in the recognition of intracellular bacterial pathogen-associated molecular patterns[29] and the two molecules share structure and functional similarities. Certain polymorphisms and

mutations in these molecules may, therefore, result in abnormalities during bacterial recognition with direct implications for CD pathogenesis. Given the importance of these results, further confirmatory studies are warranted in more and larger IBD populations. In order to maximize the opportunities to compare clinical subgroups, location was kept simple and genotyping was specifically blinded to clinical status. Mutations of the *NOD2* gene were rare among our patients with disease limited to the colon (L2). This is in accordance with recent studies showing that *NOD2* mutations (particularly L1007finsC) are strongly related to an increased risk of developing ileal CD. In our cohort of patients we only found this association after combining ileal and ileocolonic patients. This could be the consequence of the low rates of limited ileal CD in our cohort of patients compared to other studies (ranging from 40% to 50% in CD patients)[25,26]. Since location remains relatively stable during the course of the disease[24], the low rates of ileal CD seen in our patients could be attributable to the impact of interobserver disagreement[30], variation of disease location among different backgrounds[31] and even differences in diagnostic techniques. The present study suggests a relationship between disease location and different Nod-like receptor molecules, with relevant clinical implications. Distinctive subcellular location, trafficking, and expression of each Nod-like receptors could be confining the association of *NOD1* and *NOD2* with location of the disease at different parts of the gastrointestinal tract. In healthy humans, *NOD2* is expressed in Paneth cells within the crypts of the small intestine but not in colonic epithelium[6]. On the other hand, colon intestinal epithelial cells constitutively express *NOD1*[32]. *NOD1* or *NOD2* prevalence in colon or ileum could also be due to the predominance of different intracellular organisms or enteroinvasive bacteria for which they are receptors[33]. Further studies are needed to better clarify this subject.

A higher genetic load of *NOD2* mutations increased the susceptibility to CD and determined an aggressive course of the disease. Although CD behavior is a dynamic process progressing towards complicated forms in 80% of patients[24], the presence of *NOD2* variants could predict a stricturing and penetrating disease[14]. In addition, *NOD2* variants have been associated with early surgery due to stenosis and with CD recurrence after surgery[13]. No association was established between the *NOD1* polymorphism and disease behavior.

When comparing these results with other published genotype/phenotype associations, potential confounding factors should be taken into account to understand the differences. Agreement in Montreal classification, modification of the phenotype during follow-up, as well as the mixture of populations in some studies could be masking the particularities of each population. Our study adds two novel approaches to previous studies. First, two functionally related genes were analyzed for the first time in the same population, and second, the association phenotype/*NOD1* genotype was established after ruling out the strong influence of *NOD2*. Although this work emphasized the importance of *NOD1* and *NOD2* on CD disease phenotype, the complexity of IBD genetics should not be ignored. Individual combinations of genetic risk factors from other molecules such as OCTN, DLG5, TUCAN, MDR1, TNF and TLRs[34-40] would depict a specific clinical picture for each CD patient.

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COMMENTS

Background

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. Genetic and environmental factors have been suggested to predispose to CD. CD association with *NOD2* mutations has been widely replicated but with remarkable heterogeneity across populations. Similarly to *NOD2*, *NOD1* has a direct function as intracellular pattern recognition molecules but detecting different substructures from bacterial peptidoglycan. The present study examines the genetic variation in *NOD1* and *NOD2* and their respective influences on the CD phenotype in a cohort of well-characterized CD patients.

Research frontiers

Individual combinations of genetic risk factors from *NOD2*, *NOD1* and other molecules, such as OCTN, TNF and TLRs, would depict a specific clinical picture for each CD patient.

Innovations and breakthroughs

This study adds two novel approaches to previous studies. First, *NOD2* and *NOD1* were analyzed for the first time in the same population and second, the association phenotype/*NOD1* genotype was established after ruling out the strong influence of *NOD2*. The present results suggest a relationship between disease location and different Nod-like receptor molecules.

Applications

This is an association study that compares the allele or genotype frequencies of two genes between affected and unaffected individuals of Crohn's disease. Exploring new gene variants associated with inflammatory bowel disease would make possible the identification of proteins located in certain pathophysiological pathways.

Terminology

NODs are cytosolic proteins that contain a nucleotide-binding oligomerization domain (NOD). As sensors of bacterial components, *NOD1* and *NOD2* are triggered by host recognition of specific motifs in bacterial peptidoglycan and, upon activation, induce the production of proinflammatory mediators.

Peer review

This is a very well written paper. Authors examined genetic variation of *NOD1* and *NOD2*, their respective influences on Crohn's disease phenotype and gene-gene interactions. This study suggests population differences in the inheritance of risk *NOD1* polymorphism and *NOD2* mutations.

REFERENCES

1 **Shanahan F**. Crohn's disease. *Lancet* 2002; **359**: 62-69
2 **Ting JP**, Davis BK. CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu Rev Immunol* 2005; **23**: 387-414
3 **Inohara**, Chamaillard, McDonald C, Nunez G. NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu Rev Biochem* 2005; **74**: 355-383
4 **Inohara N**, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nunez G. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 2003; **278**: 5509-5512

5 **Gutierrez O**, Pipaon C, Inohara N, Fontalba A, Ogura Y, Prosper F, Nunez G, Fernandez-Luna JL. Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. *J Biol Chem* 2002; **277**: 41701-41705

6 **Ogura Y**, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, Nunez G. Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 2003; **52**: 1591-1597

7 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603

8 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606

9 **Kanazawa N**, Okafuji I, Kambe N, Nishikomori R, Nakata-Hizume M, Nagai S, Fuji A, Yuasa T, Manki A, Sakurai Y, Nakajima M, Kobayashi H, Fujiwara I, Tsutsumi H, Utani A, Nishigori C, Heike T, Nakahata T, Miyachi Y. Early-onset sarcoidosis and CARD15 mutations with constitutive nuclear factor-kappaB activation: common genetic etiology with Blau syndrome. *Blood* 2005; **105**: 1195-1197

10 **Miceli-Richard C**, Lesage S, Rybojad M, Prieur AM, Manouvrier-Hanu S, Hafner R, Chamaillard M, Zouali H, Thomas G, Hugot JP. CARD15 mutations in Blau syndrome. *Nat Genet* 2001; **29**: 19-20

11 **Ahmad T**, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002; **122**: 854-866

12 **Lesage S**, Zouali H, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; **70**: 845-857

13 **Alvarez-Lobos M**, Arostegui JI, Sans M, Tassies D, Plaza S, Delgado S, Lacy AM, Pique JM, Yague J, Panes J. Crohn's disease patients carrying Nod2/CARD15 gene variants have an increased and early need for first surgery due to stricturing disease and higher rate of surgical recurrence. *Ann Surg* 2005; **242**: 693-700

14 **Helio T**, Halme L, Lappalainen M, Fodstad H, Paavola-Sakki P, Turunen U, Farkkila M, Krusius T, Kontula K. CARD15/NOD2 gene variants are associated with familially occurring and complicated forms of Crohn's disease. *Gut* 2003; **52**: 558-562

15 **Bonen DK**, Cho JH. The genetics of inflammatory bowel disease. *Gastroenterology* 2003; **124**: 521-536

16 **Cavanaugh J**. NOD2: ethnic and geographic differences. *World J Gastroenterol* 2006; **12**: 3673-3677

17 **Satsangi J**, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996; **14**: 199-202

18 **McGovern DP**, Hysi P, Ahmad T, van Heel DA, Moffatt MF, Carey A, Cookson WO, Jewell DP. Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease. *Hum Mol Genet* 2005; **14**: 1245-1250

19 **Fritz JH**, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol* 2006; **7**: 1250-1257

20 **Hysi P**, Kabesch M, Moffatt MF, Schedel M, Carr D, Zhang Y, Boardman B, von Mutius E, Weiland SK, Leupold W, Fritzsch C, Klopp N, Musk AW, James A, Nunez G, Inohara N, Cookson WO. NOD1 variation, immunoglobulin E and asthma. *Hum Mol Genet* 2005; **14**: 935-941

21 **Tremelling M**, Hancock L, Bredin F, Sharpstone D, Bingham SA, Parkes M. Complex insertion/deletion polymorphism in NOD1 (CARD4) is not associated with inflammatory bowel disease susceptibility in East Anglia panel. *Inflamm Bowel Dis* 2006; **12**: 967-971

22 **Van Limbergen J**, Russell RK, Nimmo ER, Torkvist L, Lees CW, Drummond HE, Smith L, Anderson NH, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Sjoqvist U, Lordal M, Farrington SM, Dunlop MG, Wilson DC, Satsangi J. Contribution of the NOD1/CARD4 insertion/deletion polymorphism +32656 to inflammatory bowel disease in Northern Europe. *Inflamm Bowel Dis* 2007; **13**: 882-889

23 **Pierik M**, Yang H, Barmada MM, Cavanaugh JA, Annese V, Brant SR, Cho JH, Duerr RH, Hugot JP, McGovern DP, Paavola-Sakki P, Radford-Smith GL, Pavli P, Silverberg MS, Schreiber S, Taylor KD, Vlietinck R. The IBD international genetics consortium provides further evidence for linkage to IBD4 and shows gene-environment interaction. *Inflamm Bowel Dis* 2005; **11**: 1-7

24 **Louis E**, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. *Gut* 2001; **49**: 777-782

25 **Mendoza JL**, Murillo LS, Fernandez L, Pena AS, Lana R, Urcelay E, Cruz-Santamaria DM, de la Concha EG, Diaz-Rubio M, Garcia-Paredes J. Prevalence of mutations of the NOD2/CARD15 gene and relation to phenotype in Spanish patients with Crohn disease. *Scand J Gastroenterol* 2003; **38**: 1235-1240

26 **Nunez C**, Barreiro M, Dominguez-Munoz JE, Lorenzo A, Zapata C, Pena AS. CARD15 mutations in patients with Crohn's disease in a homogeneous Spanish population. *Am J Gastroenterol* 2004; **99**: 450-456

27 **Economou M**, Trikalinos TA, Loizou KT, Tsianos EV, Ioannidis JP. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. *Am J Gastroenterol* 2004; **99**: 2393-2404

28 **Zouali H**, Lesage S, Merlin F, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Christensen S, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Chamaillard M, Thomas G, Hugot JP. CARD4/NOD1 is not involved in inflammatory bowel disease. *Gut* 2003; **52**: 71-74

29 **Inohara N**, Ogura Y, Chen FF, Muto A, Nunez G. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem* 2001; **276**: 2551-2554

30 **Oefferlbauer-Ernst A**, Miehsler W, Eckmullner O, Travis S, Waldhoer T, Dejaco C, Gangl A, Vogelsang H, Reinisch W. Impact of interobserver disagreement on phenotype-genotype associations in Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 156-163

31 **Cho JH**, Brant SR. Genetics and genetic markers in inflammatory bowel disease. *Curr Opin Gastroenterol* 1998; **14**: 283-288

32 **Kim JG**, Lee SJ, Kagnoff MF. Nod1 is an essential signal transducer in intestinal epithelial cells infected with bacteria that avoid recognition by toll-like receptors. *Infect Immun* 2004; **72**: 1487-1495

33 **Girardin SE**, Tournebize R, Mavris M, Page AL, Li X, Stark GR, Bertin J, DiStefano PS, Yaniv M, Sansonetti PJ, Philpott DJ. CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive Shigella flexneri. *EMBO Rep* 2001; **2**: 736-742

34 **Brant SR**, Panhuysen CI, Nicolae D, Reddy DM, Bonen DK, Karaliukas R, Zhang L, Swanson E, Datta LW, Moran T, Ravenhill G, Duerr RH, Achkar JP, Karban AS, Cho JH. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. *Am J Hum Genet* 2003; **73**: 1282-1292

35 **Franchimont D**. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. *Ann N Y Acad Sci* 2004; **1024**: 124-137

36 **Pierik M**, Joossens S, Van Steen K, Van Schuerbeek N,

Vlietinck R, Rutgeerts P, Vermeire S. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis* 2006; **12**: 1-8

37 **McGovern DP**, Butler H, Ahmad T, Paolucci M, van Heel DA, Negoro K, Hysi P, Ragoussis J, Travis SP, Cardon LR, Jewell DP. TUCAN (CARD8) genetic variants and inflammatory bowel disease. *Gastroenterology* 2006; **131**: 1190-1196

38 **Negoro K**, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Satoh J, Shimosegawa T, Toyota T. Crohn's disease is associated with novel polymorphisms in the 5'-flanking region of the tumor necrosis factor gene. *Gastroenterology* 1999; **117**: 1062-1068

39 **Peltekova VD**, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36**: 471-475

40 **Rioux JD**, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001; **29**: 223-228

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RAPID COMMUNICATION

An optimized ^{13}C-urea breath test for the diagnosis of *H pylori* infection

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Abstract

AIM: To validate an optimized ^{13}C-urea breath test (^{13}C-UBT) protocol for the diagnosis of *H pylori* infection that is cost-efficient and maintains excellent diagnostic accuracy.

METHODS: 70 healthy volunteers were tested with two simplified ^{13}C-UBT protocols, with test meal (Protocol 2) and without test meal (Protocol 1). Breath samples were collected at 10, 20 and 30 min after ingestion of 50 mg ^{13}C-urea dissolved in 10 mL of water, taken as a single swallow, followed by 200 mL of water (pH 6.0) and a circular motion around the waistline to homogenize the urea solution. Performance of both protocols was analyzed at various cut-off values. Results were validated against the European protocol.

RESULTS: According to the reference protocol, 65.7% individuals were positive for *H pylori* infection and 34.3% were negative. There were no significant differences in the ability of both protocols to correctly identify positive and negative *H pylori* individuals. However, only Protocol 1 with no test meal achieved accuracy, sensitivity, specificity, positive and negative predictive values of 100%. The highest values achieved by Protocol 2 were 98.57%, 97.83%, 100%, 100% and 100%, respectively.

CONCLUSION: A 10 min, 50 mg ^{13}C-UBT with no test meal using a cut-off value of 2-2.5 is a highly accurate test for the diagnosis of *H pylori* infection at a reduced cost.

Key words: *H pylori*; ^{13}C-urea breath test; Diagnosis; Accuracy; Cost

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INTRODUCTION

H pylori infection is present in around 50% of the world population[1], with higher prevalence rates in developing countries where it is the most frequent chronic infection in human kind[2].

H pylori infection has been associated with the pathogenesis of gastric disorders such as gastritis, duodenal and gastric ulcer, gastric cancer and MALT lymphoma[3], and a variety of extradigestive disorders including hematologic, such as iron deficiency anemia[4], pernicious anemia[5], autoimmune neutropenia[6], Schönlein-Henoch purpura[7], thrombotic thrombocytopenic purpura[8] and idiopathic thrombocytopenic purpura[9]. It has also been implicated in the pathogenesis of traditional autoimmune diseases, including rheumatoid arthritis[10], Sjögren syndrome[11] and autoimmune thyroiditis[12], dermatologic diseases such as rosacea[13] and urticaria[14], and cardiovascular events[15,16] among others.

Diagnosis of *H pylori* infection can be established by either invasive or non-invasive techniques. Invasive techniques, by means of endoscopy, are expensive[17], cause patient discomfort and introduce the risk of cross-infection[18,19]; moreover, there is morbidity and mortality associated with the procedure[20] and is not indicated in all cases where the *H pylori* status must be determined[21,22]. Non-invasive methods include serology[23] ^{14}C-urea or ^{13}C-urea breath test (UBT)[24,25], stool antigen test[26] and blood urea test[27].

The principle of the ^{13}C-UBT relies upon the ability of the urease, produced by *H pylori* in the gastric mucosa, to hydrolyze the orally administered ^{13}C-urea. This enzyme breaks down any urea in the stomach to ammonia and carbon dioxide (CO_2), which is absorbed into the blood stream and then released from the lungs. The labelled carbon dioxide ($^{13}CO_2$) is detected in breath samples[28].

The aim of the present study was to standardize and validate an assay that is cost-effective, while preserving excellent diagnostic accuracy. Two simple protocols were validated against the standard European protocol[29], which included modifications in the dose, formulation and *via* of urea administration, sample collection times and test meal. Appropriate cut-off values for these assays were also established.

MATERIALS AND METHODS

Subjects

The study population included 70 volunteers with no gastrointestinal symptoms. The volunteers were informed about the study and the tests, and signed an informed consent in accordance with the Helsinki Declaration[30]. The study was classified as a research study with no biological, physiological, psychological or social risks by the Health Ministry of Colombia[31]. Because of the nature of the study in healthy volunteers, it was considered non-ethical to perform invasive tests such as biopsy, culture or endoscopy.

Protocols

Reference protocol: *H pylori* infection status of individuals was determined by the ^{13}C-UBT, according to the European protocol described before[29] and using commercial kits (TAU-KIT, Isomed SL, Madrid, Spain) that provide both a sensitivity and specificity close to 100%[25]. This protocol was standardized and was validated for our region, with over 15000 assays performed, and used as the gold standard. The ^{13}C-UBT was analyzed by means of continuous flow-isotope ratio mass spectrometry (ABCA, SerCon, Cheshire, UK) at the Laboratorio Clínico Hematológico® in Medellín, Colombia.

The reference protocol was performed as follows: After fasting for at least 8 h, individuals were given 4.2 g of citric acid dissolved in 200 mL of water. Ten minutes later, a duplicate basal breath sample was collected. Immediately after, individuals were given 100 mg of ^{13}C-urea dissolved in 125 mL of water. After 30 min, a duplicate post-urea breath sample was collected. Results over 2.5 delta-over-baseline (DOB) were considered positive for *H pylori* infection.

Protocol 1: After fasting for at least 8 h, a first basal breath sample was collected. Individuals were given 50 mg of ^{13}C-urea (99%, Isotec, Miamisburg, Ohio, USA) dissolved in 10 mL of water, taken as a single swallow. Immediately after, individuals were given 200 mL of water (pH 6.0). Volunteers, with a final volume of 210 mL, were asked to make a circular motion around the waistline for a few times to homogenize the aqueous solution and allow contact of the ^{13}C-urea with the entire gastric mucosa. Additional breath samples were collected afterwards at 10, 20 and 30 min.

Protocol 2: Same as Protocol 1, except that 4.2 g of dehydrated citric acid were added to the 200 mL of water.

The performance of both protocols was analyzed at various cut-off values from 0.5 to 5.5, at the different time intervals (10, 20 and 30 min).

Statistical analysis

The χ^2 test was used to analyze associations between qualitative variables. For quantitative variables, the Wilcoxon's signed rank sum tests and Student's *t*-test were applied. Normality of the distribution of the data was assessed with the Wilk-Shapiro test. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, Youden index, likelihood ratios for a positive (LR+ve) or negative (LR-ve) test were calculated against the defined gold standard. The effectiveness of each protocol was evaluated by ROC analysis. Processing and analysis of data were done with the SPSS (Statistical Product for Service Solutions) version 12.0 and EPIDATE Version 3.0. A value of $P < 0.05$ was considered statistically significant.

RESULTS

This study included 70 individuals, 24 (34.3%) males and 46 (65.7%) females, with an average age of 39.63 (SD ± 12.58) years for males and 34.33 (SD ± 10.17) years for females. There were no significant differences between the mean age for males and females ($P = 0.061$). According to the reference protocol, 46 (65.7%) individuals were positive for *H pylori* infection and 24 (34.3%) were negative. When assessed by gender, 17 (70.8%) males and 29 (63%) females were positive for *H pylori*; this association was not statistically significant ($P = 0.515$).

Table 1 shows the performance of the protocols in terms of sensitivity, specificity, accuracy, positive and negative predictive values, Youden index and likelihood ratios for a positive (LR+ve) or (LR-ve) test with the different DOB cut-off values at 10, 20 and 30 min. Only Protocol 1 (with no test meal) achieved accuracy, sensitivity, specificity, positive and negative predictive values of 100%. The highest values achieved by Protocol 2 were 98.57%, 97.83%, 100%, 100% and 100%, respectively.

There were no significant differences in the ability of both protocols to correctly identify positive and negative *H pylori* individuals at 10 ($P = 0.32$), 20 ($P = 0.32$) and 30 min ($P = 0.32$). These results were confirmed by ROC analysis (Figure 1). The areas under the ROC curves for both protocols were as follows: for Protocol 1, 1.0 at 10, 20 and 30 min; for Protocol 2, 0.9837 at 10 and 30 min, and 0.9873 at 20 min. Although these results were not statistically different, Protocol 1 shows the maximum optimal values for an assay.

Table 2 shows the distribution of the DOB values at 10, 20, 30 min for *H pylori* positive and negative individuals for Protocols 1 and 2. For Protocol 1, the median DOB for *H pylori* infected individuals at 10 min was 13.64, while for Protocol 2 was 12.02. There was no statistically significant difference between these 2 values (Wilcoxon, $P = 0.121$). In contrast, median DOB values at 20 and 30 min for both protocols showed significant differences ($P = 0.006$ and $P = 0.001$, respectively). In addition, for non-infected individuals there were no statistically significant differences in the median DOB values at 10, 20 and 30 min ($P = 0.710$, $P = 0.440$ and $P = 0.346$, respectively) between both protocols.

DISCUSSION

The ^{13}C-UBT has become the gold standard of the non-invasive tests for diagnosing *H pylori* infection, before and after eradication treatment. Recently, The Maastricht Ⅲ Consensus Report has recommended the ^{13}C-UBT as the best option to establish the diagnosis of *H pylori* infection, especially in patients in whom endoscopy is not indicated[22].

Table 1 Performance of protocols (P1 and P2) in terms of sensitivity, specificity, accuracy, positive and negative predictive values, Youden index and likelihood ratios for a positive (LR+ve) or (LR-ve) test with the different DOB cut-off values at 10, 20 and 30 min

Time (min)	DOB	Sensitivity		Specificity		Accuracy		Positive predictive value		Negative predictive value		Youden index		LR+ve		LR-ve	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10	0.5	100	97.83	45.83	62.5	81.43	85.71	77.97	83.33	100	93.75	0.46	0.6	1.85	2.61	[1]	0.03
	1.0	100	97.83	83.33	79.17	94.29	91.43	92	90.0	100	95	0.83	0.77	6.00	4.7	[1]	0.03
	1.5	100	97.83	95.83	95.83	98.57	97.14	97.87	97.83	100	95.83	0.96	0.94	24.00	23.48	[1]	0.02
	2.0	100	97.83	100	100	100	98.57	100	100	100	96	1.00	0.98	[2]	[2]	[1]	0.02
	2.5	100	95.65	100	100	100	97.14	100	100	100	92.31	1.00	0.96	[2]	[2]	[1]	0.04
	3.0	97.83	95.65	100	100	98.57	97.14	100	100	96.0	92.31	0.98	0.96	[2]	[2]	0.02	0.04
	3.5	97.83	95.65	100	100	98.57	97.14	100	100	96.0	92.31	0.98	0.96	[2]	[2]	0.02	0.04
	4.0	95.65	95.65	100	100	97.14	97.14	100	100	92.31	92.31	0.96	0.96	[2]	[2]	0.04	0.04
	4.5	93.48	95.65	100	100	95.71	97.14	100	100	88.89	92.31	0.93	0.96	[2]	[2]	0.07	0.04
	5.0	86.96	95.65	100	100	91.43	97.14	100	100	80	92.31	0.87	0.96	[2]	[2]	0.13	0.04
	5.5	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	[2]	[2]	0.15	0.04
20	0.5	100	97.83	66.67	62.5	88.57	85.71	85.19	83.33	100	93.75	0.67	0.6	3.00	2.61	[1]	0.03
	1.0	100	97.83	95.83	75	98.57	90	97.87	88.24	100	94.74	0.96	0.73	24.00	3.91	[1]	0.03
	1.5	100	97.83	100	83.33	100	92.86	100	91.84	100	95.24	1.00	0.81	[2]	5.87	[1]	0.03
	2.0	100	97.83	100	100	100	98.57	100	100	100	96	1.00	0.98	[2]	[2]	[1]	0.02
	2.5	100	95.65	100	100	100	97.14	100	100	100	92.31	1.00	0.96	[2]	[2]	[1]	0.04
	3.0	97.83	95.65	100	100	98.57	97.14	100	100	96	92.31	0.98	0.96	[2]	[2]	0.02	0.04
	3.5	95.65	95.65	100	100	97.14	97.14	100	100	92.31	92.31	0.96	0.96	[2]	[2]	0.04	0.04
	4.0	89.13	95.65	100	100	92.86	97.14	100	100	82.76	92.31	0.89	0.96	[2]	[2]	0.11	0.04
	4.5	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	[2]	[2]	0.15	0.04
	5.0	82.61	95.65	100	100	88.57	97.14	100	100	75	92.31	0.83	0.96	[2]	[2]	0.17	0.04
	5.5	82.61	93.48	100	100	88.57	95.71	100	100	75	88.89	0.83	0.93	[2]	[2]	0.17	0.07
30	0.5	100	97.83	54.17	45.83	84.29	80	80.7	77.59	100	91.67	0.54	0.44	2.18	1.81	[1]	0.05
	1.0	100	97.83	91.67	70.83	97.14	88.57	95.83	86.54	100	94.44	0.92	0.69	12.00	3.35	[1]	0.03
	1.5	100	97.83	100	83.33	100	92.86	100	91.84	100	95.24	1.00	0.81	[2]	5.87	[1]	0.03
	2.0	95.65	97.83	100	91.67	97.14	95.71	100	95.74	92.31	95.65	0.96	0.89	[2]	11.74	0.04	0.02
	2.5	91.3	97.83	100	100	94.29	98.57	100	100	85.71	96	0.91	0.98	[2]	[2]	0.09	0.02
	3.0	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	[2]	[2]	0.15	0.04
	3.5	82.61	95.65	100	100	88.57	97.14	100	100	75	92.31	0.83	0.96	[2]	[2]	0.17	0.04
	4.0	78.26	95.65	100	100	85.71	97.14	100	100	70.59	92.31	0.78	0.96	[2]	[2]	0.22	0.04
	4.5	78.26	95.65	100	100	85.71	97.14	100	100	70.59	92.31	0.78	0.96	[2]	[2]	0.22	0.04
	5.0	76.09	95.65	100	100	84.29	97.14	100	100	68.57	92.31	0.76	0.96	[2]	[2]	0.24	0.04
	5.5	71.74	93.48	100	100	81.43	95.71	100	100	64.86	88.89	0.72	0.93	[2]	[2]	0.28	0.07

DOB: Delta-over-baseline; [1]: ≈ 0; [2]: Φ +.

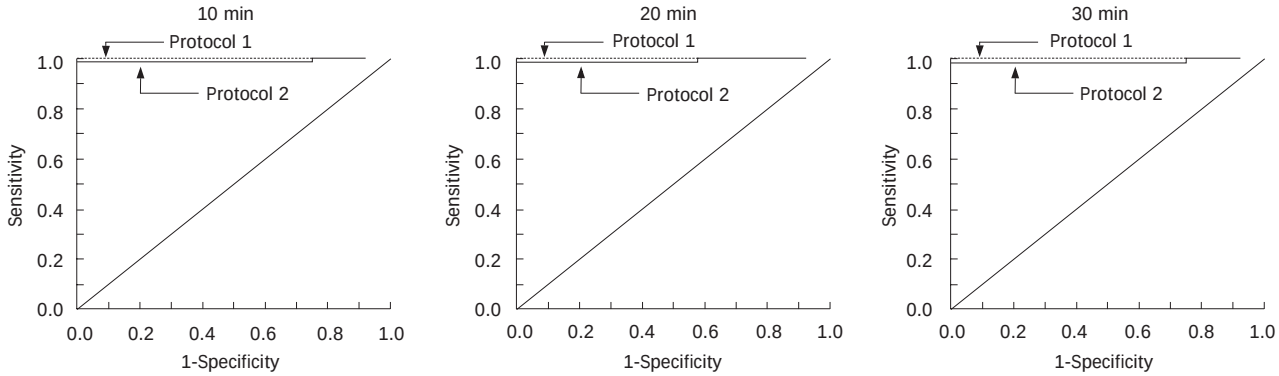

Figure 1 ROC curves for protocols 1 and 2 at 10, 20 and 30 min to establish the diagnosis of *H pylori* infection.

Table 3 shows a selection of 40 studies from the literature where relevant variations to the original ^{13}C-UBT protocol[28] have been implemented. From each study, the protocol with the best diagnostic performance was selected[32-71]. Of these, 12 (30%) yielded sensitivities and specificities of 100%[33,37,43,45,47,56,61,63,65,67-69].

Based on the results after reviewing the literature, the present study introduced several variations to simplify even further the technique and make it more cost-efficient, without compromising the high standards of sensitivity, specificity, positive and negative predictive values of the test. Below is a brief review of the evolution of the assay, since its first description, which led to the designing of the protocols evaluated in the present study.

Urea dose

Originally, the ^{13}C-UBT was described with a dose of ^{13}C-urea of 5 mg/kg of bodyweight[28]. Later on, doses of

Table 2 Distribution of DOB values in *H pylori* positive and negative individuals for both protocols at 10, 20 and 30 min

H pylori-positive individuals						
	10 min		20 min		30 min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Mean	17.38	17.69	18.25	22.32	15.44	22.08
Median	13.64	12.02	12.63	17.07	10.98	17.50
SD	14.47	12.68	22.80	15.01	17.75	13.00
H pylori-negative individuals						
	10 min		20 min		30 min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Mean	0.32	0.33	0.11	0.45	0.21	0.62
Median	0.51	0.32	0.28	0.34	0.38	0.59
SD	0.91	0.8	0.77	0.86	0.84	0.91

DOB: Delta-over-baseline.

125[35,72] and 100 mg[36,40,47,48,51,54,57,60,62,65,73-83] were validated by several American, European and Asian groups, and more recently 75 mg[32,37,43-45,49,50,55,72,84-93], 50 mg[52,56,58,94,95], 38 mg[96], 25 mg and even 10 mg[69] of ^{13}C-urea have proved to be sufficient.

Test meal

From the beginning, there has been a belief that a delay in gastric emptying is necessary for optimal performance of the test, to allow enough time for the *H pylori*-urease to react in the gastric mucosa, if present. Initially individuals were given a meal consisting of one can of "Sustacal" pudding or 120 mL of 25% glucose polymer, followed 10 min later by a polycose solution containing the ^{13}C-urea[28]. Through the years there have been numerous modifications to the test meal, including the use of citric acid alone before administering the urea[37,62,67,79,84,85,97], or mixed with the ^{13}C-urea at the time of administration[43-45,88,91,98], or as a presentation in combination with the ^{13}C-urea[42,61,72,94,99]. Several alternatives to citric acid have also been tested, including orange juice[43,49,67,90,95] and apple juice[100], as well as other types of food such as milk[48,58,101,102] and a pudding test meal[34,46], and even water[41,103]. As shown in Table 3, the majority of the test meals have provided reliable results. Even the complete absence of a test meal has shown little, if any, variation in the diagnostic performance of the assay[35,40,47,53,56,59,60,102,104].

Via of administration and formulation of ^{13}C-urea

Another issue that has been addressed by different groups is the interference of other urease-producing bacteria in the oral cavity and oropharynx[105,106], leading to an increase in false positive values. As a result, there have been different approaches in the formulation and way of administration of the labeled urea, including the development of ^{13}C-urea tablets[42,61,63,64,95], capsules[68,96,99,107], and even the intragastric instillation of the urea through the endoscope[80,108,109]. Some have also suggested mouth rinsing before and after urea administration[39-41,48,51,52,54,58,60,68,110].

Sample collection times

The ^{13}C-UBT was originally described with a basal sample and 18 post-urea samples taken during the following 180 min[28]. Rapidly the assay was modified and currently only 2 samples are obtained: pre and post-urea. Sampling times, although shorter than initially, have differed among protocols.

Ways of reducing the cost of the ^{13}C-UBT could include decreasing the amount of ^{13}C-urea used, reducing the duration of the test, and improving the ease with which the test can be administered and tolerated. The conventional European ^{13}C-UTB protocol used in our region is sensitive and specific enough (values close to 100%), but it takes 40-45 min to complete and is performed using 100 mg of ^{13}C-urea. For the present study we decided to use 50 mg of ^{13}C-urea to reduce the cost of the assays by half, a dose that has proved to be as accurate as higher doses[52,56,58,61,63,68]. The ^{13}C-urea was administered diluted in 10 mL of water and taken as a single swallow, to try to avoid cross-contamination with urease-producing oropharyngeal bacteria. Immediately after 200 mL of water (pH 6.0) with 4.2 g of citric acid (Protocol 2) and without citric acid (Protocol 1) were administered, and volunteers were asked to make a circular motion around the waistline for a few times to homogenize the aqueous solution and allow contact of the ^{13}C-urea with the entire gastric mucosa. It has been shown that *H pylori* urease operates in a pH range from 3.1 to 10, with an optimal activity at pH 6.0[111,112]. By utilizing water at pH 6.0, activity of the *H pylori* urease was optimized for Protocol 1, where no citric acid was used. Acid solutions have been used by many to delay gastric emptying and to provide a higher acidic environment to induce *H pylori*-urease activity[43,98], although it has been demonstrated by Pantoflickova *et al*[100] that the emptying is determined by the caloric density of the test meal rather than by its pH. Finally, in order to reduce the duration of the test, both protocols were tested at different sampling times: 10, 20 and 30 min.

This study included 70 individuals, 34.3% males and 65.7% females, with an average age of 39.63 ± 12.58 years for males and 34.33 ± 10.17 years for females. According to the reference protocol, 46 (65.7%) individuals were positive for *H pylori* infection. No statistically significant association was found between gender and presence of *H pylori* infection ($P = 0.515$).

There were no significant differences in the ability of both protocols to correctly identify positive and negative *H pylori* individuals at the various sampling times. However, only Protocol 1, with no test meal, yielded a test with sensitivity, specificity, positive and negative predictive values, and accuracy of 100% when compared to the gold standard, when using a DOB cut-off value between 2 and 2.5 at 10 and 20 min, and a DOB cut-off value of 1.5 at 20 and 30 min. For Protocol 2, with citric acid, the highest accuracy (98.57%) was achieved at 10 min using a DOB cut-off value of 2.0, at 20 min a DOB cut-off value of 2.0, and at 30 min with a DOB of 2.5.

Median DOB for *H pylori* infected individuals at 10 min was 13.64, while for Protocol 2 was 12.02. There was no statistically significant difference between these 2 values (Wilcoxon, $P = 0.121$). However, median DOB values at 20 and 30 min for both protocols showed significant differences ($P = 0.006$ and $P = 0.001$, respectively). These results are in accordance with those by Atherton *et al*[113]

Table 3 ^{13}C-UBT protocol with best diagnostic performance from each study with samples obtained within 30 min of ^{13}C-urea administration: Review of literature

First author (reference)	Year	Measuring equipment	Gold standard	*n*	Pre-analytical	^{13}C-urea dose (mg)	^{13}C-urea formulation and via of administration	Test meal	Additional information related to ^{13}C-urea administration	*t*	Cut-off point (DOB)	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Acc. (%)
Braden[32]	1994	IRMS	^{13}C-UBT	217	Overnight fasting	75	NA	None		20	5	99	100			
Koletzko[33]	1995	IRMS, NDIRS	^{13}C-UBT	51	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with ^{13}C-urea		15	5	100	100			
Klein[34]	1996	IRMS	H	465	NA	125	Powder in 90 mL sterile water (Meretek kit)	Ensure		30	2.4	95.4	87.9			94.8
Malaty[35]	1996	IRMS	H, RUT, C	66	Overnight fasting	125	Powder in 100 mL water	None		20	2.4	96	100			
Taniguchi[36]	1996	NDIRS	H	153	Overnight fasting	100	Powder in 30 mL water	None		15	1	97.8	74			
Domínguez-Muños[37]	1997	IRMS	H, RUT, C	80	Overnight fasting	80	Powder in 50 mL water	200 mL 0.1 mol/L citric acid solution		30	4	100	100			
Epple[38]	1997	IRMS	H	77	Overnight fasting	75	NA	Citric acid		30	1.3	96	100			
Kato[39]	1998	IRMS	H, C, RUT	133	Overnight fasting	100	Powder in 100 mL of water	None	Mouth rinsing after ^{13}C-urea	10	3.5	99	100			
Miwa[40]	1998	IRMS	H	409	8 h fasting	100	Powder	None	Mouth rinsing before and after ^{13}C-urea	20	5	97	97			
Ohara[41]	1998	IRMS	H, RUT, C	248	Overnight fasting	100	Powder in 100 mL tap water	None	Mouth rinsing after ^{13}C-urea	20	2.5	98	98			98
Hamlet[42]	1999	IRMS	^{13}C-UBT, H, RUT, C	134	Overnight fasting	100	Two tablets (Diabact UBT) with 50 mg of ^{13}C-urea + 456 mg of anhydrous citric acid swallowed with 200 mL of water	Taken with ^{13}C-urea		10	1.8	95	100			
Leodolter[43]	1999	IRMS	H, RUT, C	50	NA	75	Powder in 200 mL 0.1 mol/L citric acid solution	Taken with ^{13}C-urea		30	4	100	100			
Leodolter[44]	1999	IRMS	H, RUT, C	233	NA	75	Powder in 200 mL citric acid solution	Taken with ^{13}C-urea		30	4	95	98			97
Savarino[45]	1999	IRMS	H, RUT	134	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with ^{13}C-urea		30	5	100	100			
Van der Hulst[46]	1999	LARA	H, C	544	NA	100	Powder in 50 mL sterile water	Ensure		30	6.3-7.5	93-95	94-96	95-98	86-94	
Gisbert[47]	2000	IRMS	^{13}C-UBT, H	53	Overnight fasting	100	Powder in 50 mL water (TAU-KIT)	None		30	3.3-3.9	100	100			
Peng[48]	2000	IRMS	H, RUT, C	136	6 h fasting	100	Powder in 50 mL sterile water	100 mL milk	Mouth rinsing after ^{13}C-urea and laid on their sides, changing sides every 5 min	15	4.8	94	89			
Riepl[49]	2000	NDIRS	H, C	100	Overnight fasting	75	Powder in 200 mL orange juice	Taken with ^{13}C-urea		15	6.5	92	94	89	94	

Savarino[50]	2000	IRMS	H, RUT	117	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with ^{13}C-urea		30	5	98	97	98	97	98
Sheu[51]	2000	IRMS	H, C	441	Overnight fasting	100	NA	100 mL of fatty test meal	Mouth rinsing before and after ^{13}C-urea	15	4	98	97			
Sheu[52]	2000	IRMS, NDIRS	H, C	177	Overnight fasting	50	NA	100 mL citric acid solution	Mouth rinsing after ^{13}C-urea	15	3.5	96	99	99	97	
Wong[53]	2000	IRMS	H, RUT	202	Overnight fasting	75	Powder in 50 mL distilled water	2.4 g of citric acid		30	5	96	98	98	96	97
Yoshida[54]	2000	LARA	H, C, PCR	104	Overnight fasting	100	Powder in 50 mL distilled water	None	Mouth rinsing after ^{13}C-urea	20	2.7	98	100			99
Mana[55]	2001	NDIRS	H	223	Overnight fasting	75	Powder	20 mL 0.1 mol/L citric acid solution		10		100	95	94	100	
Wong[56]	2001	IRMS	H, RUT	101	Overnight fasting	75	Powder in 50 mL water	None		30	3.5-4.5	100	100			100
Chua[57]	2002	IRMS	H, RUT, S	100	NA	100	Powder in solution containing citric acid and ^{13}C-urea		Pacients laid on their left side for 30 min	30	3.5	94	100	100	89	
Liao[58]	2002	IRMS	H, RUT	152	Overnight fasting	50	Powder in 50 mL sterile water	200 mL full-cream cow's milk	Patients gargled with water 3 times after ^{13}C-urea and laid on their sides, changing sides every 3 min	15	2.5-3.0	99	97	99	97	99
Ng[59]	2002	IRMS	H, RUT	123	Regular meal within 2 h of the ^{13}C-urea	75	Powder in 50 mL water	2.4 g citric acid in 200 mL solution		30	5.5	93	97	100	97	
Chen[60]	2003	NDIRS	H, RUT, C, SAT	586	Overnight fasting	100	Powder in 100 mL of water	None	Patients gargled with water 3 times after ^{13}C-urea and laid down on the left side for 5 min	20	3.5	98	97			98
Gatta[61]	2003	IRMS	H, RUT, C	200	Overnight fasting	50	Tablet (Diabact UBT) with 50 mg of ^{13}C-urea and 456 mg of anhydrous citric acid swallowed with 200 mL of water	Citric acid		10	1.65-3.15	100	100			
Gisbert[62]	2003	IRMS	H, RUT	36	Overnight fasting	100	Powder in 50 mL water (TAU-KIT)	200 mL solution with 4.2 g citric acid		30	5	96	100	100	91	
Wong[63]	2003	IRMS	H, RUT	150	Overnight fasting	50	Tablet (Diabact UBT) with 50 mg of ^{13}C-urea and 456 mg of anhydrous citric acid swallowed with 200 mL of water	Citric acid		20	2.1	100	100			
Ohara[64]	2004	IRMS	^{13}C-UBT, H, C, RUT	254	Overnight fasting	100	Film-coated tablet swallowed with 100 mL of water	None		20	2.5	98	98			98

Urita[65]	2004	IRMS	H, S	129	Overnight fasting	100	Powder in 100 mL tap water	None	Sample taken through nostril	20	2.5	100	100			100
Beiki[66]	2005	NDIRS	^{14}C-UBT, H, RUT	76	Overnight fasting	75	Powder in 200 mL orange juice			30	3.5	100	97	98	100	99
Kopacova[67]	2005	IRMS	^{13}C-UBT	27	Overnight fasting	100	Powder in 50 mL distilled water with 1 g citric acid	150 mL distilled water with 3 g citric acid, orange juice or distilled water		10	3.5	100	100			100
Peng[68]	2005	IRMS	H, RUT, C	50	6 h fasting	100	Capsule with water	None	Mouth rinsing before and after ^{13}C-urea and laid on their sides, changing sides every 5 min	15	4-5	100	100			100
Gatta[69]	2006	IRMS	H, RUT	100	Overnight fasting	25	Dissolved in water	Citric acid (1 g)		30	4.4-6.26	100	100			
Mauro[70]	2006	IRMS	H, C	176	Overnight fasting	75	Powder in 100 mL citric acid solution	Taken with ^{13}C-urea		30	3	100	99	95-98	100	
Mauro[71]	2006	IRMS	H, C	67	Overnight fasting	75	Powder in 100 mL citric acid solution	Taken with ^{13}C-urea		10	3	100	96	95-98	99-100	
Present study	2007	IRMS	^{13}C-UBT	70	Overnight fasting	50	Powder in 10 mL sterile water immediately followed by 200 mL sterile water	None	Patients made a circular motion around the waistline for a few times	10	2.0-2.5	100	100	100	100	100

n: Participating individuals; *t*: Sampling time; PPV: Positive predictive value; NPV: Negative predictive value; Acc: Accuracy; UBT: Urea breath test; H: histology; C: Culture; RUT: Rapid urease test; S: Serology; NA: Not available; IRMS: Isotope ratio mass spectrometry; NDIRS: Non-dispersive infrared spectrometry; LARA: Laser assisted ratio analyser; DOB: Delta-over-baseline.

and Gisbert *et al*[47], who showed that the test meal did not affect ^{13}C-UBT results at 10 min, but increased the values thereafter.

In conclusion, an optimal laboratory test should be non-invasive, easy to perform, highly reproducible, cost-efficient and with a sensitivity and specificity close to 100%. When compared to other protocols published in the literature, the present conditions of Protocol 1 have further optimized the ^{13}C-UBT assay, as this is the only protocol with a sampling time of 10 min, a ^{13}C-urea dose of 50 mg and no test meal that can yield a test with 100% accuracy for the diagnosis of *H pylori* infection. These variations provide a protocol that can reduce the cost of the ^{13}C-UBT assay, is innocuous, well tolerated, has no restrictions and could be implemented for all patients in whom endoscopy is not an indication[21,22] and as a screening test for *H pylori* epidemiological studies. Further studies are underway to try to decrease the ^{13}C-urea to an even lower dose, using biopsy as the gold standard.

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COMMENTS

Background

H pylori infection is present in around 50% of the world population and has been associated with the pathogenesis of gastric disorders such as gastritis, gastric ulcer and MALT lymphoma, and a variety of extradigestive diseases, including idiopathic thrombocytopenic purpura, iron deficiency anemia and autoimmune thyroiditis, among others. Diagnosis of *H pylori* infection can be established by either invasive techniques, by means of endoscopy, or non-invasive techniques such as the ^{13}C-urea breath test.

Research frontiers

The ^{13}C-urea breath test relies upon the ability of an enzyme (urease), produced by *H pylori* in the stomach, to break down the administered urea. Patients swallow the urea labelled with a non-radioactive isotope (^{13}C). After a few minutes, the isotope-labelled carbon dioxide ($^{13}CO_2$) is exhaled in the breath if there is presence of *H pylori* urease in the stomach. The difference in the $^{13}CO_2$ values before and after ingestion of the labelled urea will determine the presence of infection.

Innovations and breakthroughs

Many have attempted to lower the high cost of the ^{13}C-urea breath test, while preserving excellent diagnostic accuracy. For this purpose, modifications in the

dose, formulation and way of administration, sample collection times and test meals have been evaluated.

Applications

A low cost ^{13}C-urea breath test for the detection of *H pylori* infection before and after eradication treatment will make this non-invasive assay more accessible for patients, especially in developing countries.

Terminology

^{13}C-UBT: Breath test that includes urea labelled with ^{13}C, a non-radioactive isotope. DOB: Delta over base line, units used to express the amount of $^{13}CO_2$ contained in the breath sample.

Peer review

This is a well written and comprehensively referenced article. The methods section is adequately described and the results clearly presented. The conclusions are a fair interpretation of the results.

REFERENCES

1 **Crowe SE**. Helicobacter infection, chronic inflammation, and the development of malignancy. *Curr Opin Gastroenterol* 2005; **21**: 32-38
2 **Weaver LT**. Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 16 February 1995. Aspects of Helicobacter pylori infection in the developing and developed world. Helicobacter pylori infection, nutrition and growth of West African infants. *Trans R Soc Trop Med Hyg* 1995; **89**: 347-350
3 **Suzuki H**, Marshall BJ, Hibi T. Overview: Helicobacter pylori and extragastric disease. *Int J Hematol* 2006; **84**: 291-300
4 **DuBois S**, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: a review of the evidence. *Am J Gastroenterol* 2005; **100**: 453-459
5 **Kaptan K**, Beyan C, Ural AU, Cetin T, Avcu F, Gulsen M, Finci R, Yalcin A. Helicobacter pylori--is it a novel causative agent in Vitamin B12 deficiency? *Arch Intern Med* 2000; **160**: 1349-1353
6 **Gupta V**, Eden AJ, Mills MJ. Helicobacter pylori and autoimmune neutropenia. *Clin Lab Haematol* 2002; **24**: 183-185
7 **Cecchi R**, Torelli E. Schonlein-Henoch purpura in association with duodenal ulcer and gastric Helicobacter pylori infection. *J Dermatol* 1998; **25**: 482-484
8 **Franchini M**. Thrombotic thrombocytopenic purpura: proposal of a new pathogenic mechanism involving Helicobacter pylori infection. *Med Hypotheses* 2005; **65**: 1128-1131
9 **Campuzano-Maya G**. Proof of an association between Helicobacter pylori and idiopathic thrombocytopenic purpura in Latin America. *Helicobacter* 2007; **12**: 265-273
10 **Zentilin P**, Seriolo B, Dulbecco P, Caratto E, Iiritano E, Fasciolo D, Bilardi C, Mansi C, Testa E, Savarino V. Eradication of Helicobacter pylori may reduce disease severity in rheumatoid arthritis. *Aliment Pharmacol Ther* 2002; **16**: 1291-1299
11 **El Miedany YM**, Baddour M, Ahmed I, Fahmy H. Sjogren's syndrome: concomitant H. pylori infection and possible correlation with clinical parameters. *Joint Bone Spine* 2005; **72**: 135-141
12 **de Luis DA**, Varela C, de La Calle H, Canton R, de Argila CM, San Roman AL, Boixeda D. Helicobacter pylori infection is markedly increased in patients with autoimmune atrophic thyroiditis. *J Clin Gastroenterol* 1998; **26**: 259-263
13 **Boixeda de Miquel D**, Vazquez Romero M, Vazquez Sequeiros E, Foruny Olcina JR, Boixeda de Miquel P, Lopez San Roman A, Aleman Villanueva S, Martin de Argila de Prados C. Effect of Helicobacter pylori eradication therapy in rosacea patients. *Rev Esp Enferm Dig* 2006; **98**: 501-509
14 **Federman DG**, Kirsner RS, Moriarty JP, Concato J. The effect of antibiotic therapy for patients infected with Helicobacter pylori who have chronic urticaria. *J Am Acad Dermatol* 2003; **49**: 861-864
15 **Mendall MA**, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, Camm AJ, Northfield TC. Relation of Helicobacter pylori infection and coronary heart disease. *Br Heart J* 1994; **71**: 437-439
16 **Kowalski M**, Pawlik M, Konturek JW, Konturek SJ. Helicobacter pylori infection in coronary artery disease. *J Physiol Pharmacol* 2006; **57** Suppl 3: 101-111
17 **Bytzer P**. Cost-effectiveness of gastroscopy. *Ital J Gastroenterol Hepatol* 1999; **31**: 749-760
18 **Nelson DB**. Infectious disease complications of GI endoscopy: Part I, endogenous infections. *Gastrointest Endosc* 2003; **57**: 546-556
19 **Nelson DB**. Infectious disease complications of GI endoscopy: part II, exogenous infections. *Gastrointest Endosc* 2003; **57**: 695-711
20 **McCloy R**. Asleep on the job: sedation and monitoring during endoscopy. *Scand J Gastroenterol Suppl* 1992; **192**: 97-101
21 **Talley NJ**, Vakil N. Guidelines for the management of dyspepsia. *Am J Gastroenterol* 2005; **100**: 2324-2337
22 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781
23 **Thijs JC**, van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, Luijt DS, Meyer BC, Kleibeuker JH. Diagnostic tests for Helicobacter pylori: a prospective evaluation of their accuracy, without selecting a single test as the gold standard. *Am J Gastroenterol* 1996; **91**: 2125-2129
24 **Logan RP**. Urea breath tests in the management of Helicobacter pylori infection. *Gut* 1998; **43** Suppl 1: S47-S50
25 **Gisbert JP**, Pajares JM. Review article: C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. *Aliment Pharmacol Ther* 2004; **20**: 1001-1017
26 **Gisbert JP**, Pajares JM. Stool antigen test for the diagnosis of Helicobacter pylori infection: a systematic review. *Helicobacter* 2004; **9**: 347-368
27 **Fry LC**, Curioso WH, Rickes S, Horton G, Hirschowitz BI, Monkemuller K. Comparison of 13C- urea blood test to 13C-breath test and rapid urease test for the diagnosis of Helicobacter pylori infection. *Acta Gastroenterol Latinoam* 2005; **35**: 225-229
28 **Graham DY**, Klein PD, Evans DJ Jr, Evans DG, Alpert LC, Opekun AR, Boutton TW. Campylobacter pylori detected noninvasively by the 13C-urea breath test. *Lancet* 1987; **1**: 1174-1177
29 **Logan RP**, Dill S, Bauer FE, Walker MM, Hirschl AM, Gummett PA, Good D, Mossi S. The European 13C-urea breath test for the detection of Helicobacter pylori. *Eur J Gastroenterol Hepatol* 1991; **3**: 915-921
30 **World Medical Association Declaration of Helsinki:** ethical principles for medical research involving human subjects. *J Int Bioethique* 2004; **15**: 124-129
31 **República de Colombia, Salud Md.** Resolución No. 008430 de 1993 (4 de octubre de 1993), por la cual se establecen las normas científicas, técnicas y administrativas para la investigación en salud. Available from: URL: http://www.unal.edu.co/dib/normas/etica.html
32 **Braden B**, Duan LP, Caspary WF, Lembcke B. More convenient 13C-urea breath test modifications still meet the criteria for valid diagnosis of Helicobacter pylori infection. *Z Gastroenterol* 1994; **32**: 198-202
33 **Koletzko S**, Haisch M, Seeboth I, Braden B, Hengels K, Koletzko B, Hering P. Isotope-selective non-dispersive infrared spectrometry for detection of Helicobacter pylori infection with 13C-urea breath test. *Lancet* 1995; **345**: 961-962
34 **Klein PD**, Malaty HM, Martin RF, Graham KS, Genta RM, Graham DY. Noninvasive detection of Helicobacter pylori infection in clinical practice: the 13C urea breath test. *Am J Gastroenterol* 1996; **91**: 690-694
35 **Malaty HM**, el-Zimaity HM, Genta RM, Klein PD, Graham DY. Twenty-minute fasting version of the US 13C-urea breath test for the diagnosis of H. pylori infection. *Helicobacter* 1996; **1**: 165-167
36 **Taniguchi Y**, Kimura K, Sohara H, Shirasaki A, Kawada H, Satoh K, Kihira K, Wang XM, Takimoto T, Goto Y, Takatori K,

Iida K, Kajiwara M. Simple 13C-urea breath test with infra-red spectrophotometer. *J Gastroenterol* 1996; **31** Suppl 9: 37-40

37 **Dominguez-Munoz JE**, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of Helicobacter pylori infection. *Gut* 1997; **40**: 459-462

38 **Epple HJ**, Kirstein FW, Bojarski C, Frege J, Fromm M, Riecken EO, Schulzke JD. 13C-urea breath test in Helicobacter pylori diagnosis and eradication. Correlation to histology, origin of 'false' results, and influence of food intake. *Scand J Gastroenterol* 1997; **32**: 308-314

39 **Kato M**, Asaka M, Kudo T, Katagiri M, Komatsu Y, Sato F, Sukegawa M, Kobayashi T, Kagaya H, Nishikawa K, Kudo M, Hokari K, Hige S, Watanabe M, Takeda H, Sugiyama T. Ten minute 13C-urea breath test for the diagnosis of Helicobacter pylori infection. *J Gastroenterol* 1998; **33** Suppl 10: 40-43

40 **Miwa H**, Murai T, Ohkura R, Nagahara A, Watanabe H, Terai T, Watanabe S, Sato N. Usefulness of the [13C]-urea breath test for detection of Helicobacter pylori infection in fasting patients. *J Gastroenterol Hepatol* 1998; **13**: 1039-1043

41 **Ohara S**, Kato M, Asaka M, Toyota T. Studies of 13C-urea breath test for diagnosis of Helicobacter pylori infection in Japan. *J Gastroenterol* 1998; **33**: 6-13

42 **Hamlet A**, Stage L, Lonroth H, Cahlin C, Nystrom C, Pettersson A. A novel tablet-based 13C urea breath test for Helicobacter pylori with enhanced performance during acid suppression therapy. *Scand J Gastroenterol* 1999; **34**: 367-374

43 **Leodolter A**, Dominguez-Munoz JE, Von Arnim U, Malfertheiner P. Citric acid or orange juice for the 13C-urea breath test: the impact of pH and gastric emptying. *Aliment Pharmacol Ther* 1999; **13**: 1057-1062

44 **Leodolter A**, Dominguez-Munoz JE, von Arnim U, Kahl S, Peitz U, Malfertheiner P. Validity of a modified 13C-urea breath test for pre- and posttreatment diagnosis of Helicobacter pylori infection in the routine clinical setting. *Am J Gastroenterol* 1999; **94**: 2100-2104

45 **Savarino V**, Mela GS, Zentilin P, Bisso G, Pivari M, Mansi C, Mele MR, Bilardi C, Vigneri S, Celle G. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for 13C-urea breath test. *Am J Gastroenterol* 1999; **94**: 1203-1208

46 **Van Der Hulst RW**, Lamouliatte H, Megraud F, Pounder RE, Stolte M, Vaira D, Williams M, Tytgat GN. Laser assisted ratio analyser 13C-urea breath testing, for the detection of H. pylori: A prospective diagnostic European multicentre study. *Aliment Pharmacol Ther* 1999; **13**: 1171-1177

47 **Gisbert JP**, Vazquez MA, Jimenez I, Cruzado AI, Carpio D, Del Castillo E, Martin MJ, Morales A, Pajares R, Rodriguez A, Pajares JM. 13C-urea breath test for the diagnosis of Helicobacter pylori infection before treatment: is citric acid necessary? *Dig Liver Dis* 2000; **32**: 20-24

48 **Peng NJ**, Hsu PI, Lee SC, Tseng HH, Huang WK, Tsay DG, Ger LP, Lo GH, Lin CK, Tsai CC, Lai KH. A 15-minute [13C]-urea breath test for the diagnosis of Helicobacter pylori infection in patients with non-ulcer dyspepsia. *J Gastroenterol Hepatol* 2000; **15**: 284-289

49 **Riepl RL**, Folwaczny C, Otto B, Klauser A, Blendinger C, Wiebecke B, Konig A, Lehnert P, Heldwein W. Accuracy of 13C-urea breath test in clinical use for diagnosis of Helicobacter pylori infection. *Z Gastroenterol* 2000; **38**: 13-19

50 **Savarino V**, Landi F, Dulbecco P, Ricci C, Tessieri L, Biagini R, Gatta L, Miglioli M, Celle G, Vaira D. Isotope ratio mass spectrometry (IRMS) versus laser-assisted ratio analyzer (LARA): a comparative study using two doses of. *Dig Dis Sci* 2000; **45**: 2168-2174

51 **Sheu BS**, Lee SC, Yang HB, Kuo AW, Wang YL, Shiesh SC, Wu JJ, Lin XZ. Selection of lower cutoff point of [13C]urea breath test is helpful to monitor H. pylori eradication after proton pump inhibitor-based triple therapy. *Dig Dis Sci* 2000; **45**: 1330-1336

52 **Sheu BS**, Lee SC, Yang HB, Wu HW, Wu CS, Lin XZ, Wu JJ. Lower-dose (13)C-urea breath test to detect Helicobacter pylori infection-comparison between infrared spectrometer and mass spectrometry analysis. *Aliment Pharmacol Ther* 2000; **14**: 1359-1363

53 **Wong WM**, Wong BC, Wong KW, Fung FM, Lai KC, Hu WH, Yuen ST, Leung SY, Lau GK, Lai CL, Chan CK, Go R, Lam SK. (13)C-urea breath test without a test meal is highly accurate for the detection of Helicobacter pylori infection in Chinese. *Aliment Pharmacol Ther* 2000; **14**: 1353-1358

54 **Yoshida H**, Hirota K, Ogura K, Maeda S, Shiratori Y, Sasaki Y, Omata M. Determination of the optimal cut-off value for the [13C]-urea breath test based on a Helicobacter pylori-specific polymerase chain reaction assay. *J Gastroenterol Hepatol* 2000; **15**: 155-160

55 **Mana F**, Franken PR, Ham HR, Urbain D. Cut-off point, timing and pitfalls of the 13C-urea breath test as measured by infrared spectrometry. *Dig Liver Dis* 2001; **33**: 30-35

56 **Wong WM**, Wong BC, Li TM, Wong KW, Cheung KL, Fung FM, Xia HH, Lam SK. Twenty-minute 50 mg 13C-urea breath test without test meal for the diagnosis of Helicobacter pylori infection in Chinese. *Aliment Pharmacol Ther* 2001; **15**: 1499-1504

57 **Chua TS**, Fock KM, Teo EK, Ng TM. Validation of 13C-urea breathtest for the diagnosis of Helicobacter pylori infection in the Singapore population. *Singapore Med J* 2002; **43**: 408-411

58 **Liao CC**, Lee CL, Chiang TC, Lee SC, Huang SH, Tu TC, Chen TK, Wu CH. The 13C-urea breath test to detect Helicobacter pylori infection: a validated simple methodology with 50 mg 13C-urea. *Aliment Pharmacol Ther* 2002; **16**: 787-792

59 **Ng FH**, Lai KC, Wong BC, Wong WM, Wong SY, Chow KC, Yuen ST, Leung SY, Lam SK. [13C]-urea breath test without prior fasting and without test meal is accurate for the detection of Helicobacter pylori infection in Chinese. *J Gastroenterol Hepatol* 2002; **17**: 834-838

60 **Chen TS**, Chang FY, Chen PC, Huang TW, Ou JT, Tsai MH, Wu MS, Lin JT. Simplified 13C-urea breath test with a new infrared spectrometer for diagnosis of Helicobacter pylori infection. *J Gastroenterol Hepatol* 2003; **18**: 1237-1243

61 **Gatta L**, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, Miglioli M, Vaira D. A rapid, low-dose, 13C-urea tablet for the detection of Helicobacter pylori infection before and after treatment. *Aliment Pharmacol Ther* 2003; **17**: 793-798

62 **Gisbert JP**, Ducons J, Gomollon F, Dominguez-Munoz JE, Borda F, Mino G, Jimenez I, Vazquez MA, Santolaria S, Gallego S, Iglesias J, Pastor G, Hervas A, Pajares JM. Validation of the 13c-urea breath test for the initial diagnosis of helicobacter pylori infection and to confirm eradication after treatment. *Rev Esp Enferm Dig* 2003; **95**: 121-126, 115-120

63 **Wong WM**, Lam SK, Lai KC, Chu KM, Xia HH, Wong KW, Cheung KL, Lin SK, Wong BC. A rapid-release 50-mg tablet-based 13C-urea breath test for the diagnosis of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2003; **17**: 253-257

64 **Ohara S**, Kato M, Saito M, Fukuda S, Kato C, Hamada S, Nagashima R, Obara K, Suzuki M, Honda H, Asaka M, Toyota T. Comparison between a new 13C-urea breath test, using a film-coated tablet, and the conventional 13C-urea breath test for the detection of Helicobacter pylori infection. *J Gastroenterol* 2004; **39**: 621-628

65 **Urita Y**, Hike K, Torii N, Kikuchi Y, Kanda E, Kurakata H, Sasajima M, Miki K. Breath sample collection through the nostril reduces false-positive results of 13C-urea breath test for the diagnosis of helicobacter pylori infection. *Dig Liver Dis* 2004; **36**: 661-665

66 **Beiki D**, Khalaj A, Dowlatabadi R, Eftekhari M, Al-Seyed Hossein MH, Fard A, Fallahi B, Khoshayand MR. Validation of 13C-urea breath test with non dispersive isotope selective infrared spectroscopy for the diagnosis of Helicobacter pylori infection: a survey in Iranian population. *DARU* 2005; **13**: 52-55

67 **Kopacova M**, Bures J, Vorisek V, Konstacky M, Rejchrt S, Zivny P, Douda T, Palicka V. Comparison of different protocols for 13C-urea breath test for the diagnosis of Helicobacter pylori infection in healthy volunteers. *Scand J Clin Lab Invest* 2005; **65**: 491-498

68 **Peng NJ**, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, Tseng HH, Huang WK, Lo GH, Hsu PI. Capsule 13C-urea breath test for the diagnosis of Helicobacter pylori infection. *World J*

Gastroenterol 2005; **11**: 1361-1364
69 **Gatta L**, Ricci C, Tampieri A, Osborn J, Perna F, Bernabucci V, Vaira D. Accuracy of breath tests using low doses of 13C-urea to diagnose Helicobacter pylori infection: a randomised controlled trial. *Gut* 2006; **55**: 457-462
70 **Mauro M**, Radovic V, Zhou P, Wolfe M, Kamath M, Bercik P, Croitoru K, Armstrong D. 13C urea breath test for (Helicobacter pylori): determination of the optimal cut-off point in a Canadian community population. *Can J Gastroenterol* 2006; **20**: 770-774
71 **Mauro M**, Radovic V, Wolfe M, Kamath M, Bercik P, Armstrong D. 13C urea breath test for (Helicobacter pylori): evaluation of 10-minute breath collection. *Can J Gastroenterol* 2006; **20**: 775-778
72 **Graham DY**, Malaty HM, Cole RA, Martin RF, Klein PD. Simplified 13C-urea breath test for detection of Helicobacter pylori infection. *Am J Gastroenterol* 2001; **96**: 1741-1745
73 **Thijs WJ**, Thijs JC, Kleibeuker JH, Elzinga H, Stellaard F. Evaluation of clinical and home performance of the 13C-urea breath test for the detection of Helicobacter pylori. *Eur J Gastroenterol Hepatol* 1995; **7**: 603-607
74 **Perez Garcia JI**, Pajares Garcia JM, Jimenez Alonso I. C13 urea breath test in the diagnosis of Helicobacter pylori infection in the gastric mucosa. Validation of the method. *Rev Esp Enferm Dig* 1996; **88**: 202-208
75 **Kato M**, Asaka M, Ohara S, Toyota T. Clinical studies of 13C-urea breath test in Japan. *J Gastroenterol* 1998; **33** Suppl 10: 36-39
76 **Lee HS**, Gwee KA, Teng LY, Kang JY, Yeoh KG, Wee A, Chua BC. Validation of [13C]urea breath test for Helicobacter pylori using a simple gas chromatograph-mass selective detector. *Eur J Gastroenterol Hepatol* 1998; **10**: 569-572
77 **Ohara S**, Kato M, Asaka M, Toyota T. The UBiT-100 13CO2 infrared analyzer: comparison between infrared spectrometric analysis and mass spectrometric analysis. *Helicobacter* 1998; **3**: 49-53
78 **Tanahashi T**, Kodama T, Yamaoka Y, Sawai N, Tatsumi Y, Kashima K, Higashi Y, Sasaki Y. Analysis of the 13C-urea breath test for detection of Helicobacter pylori infection based on the kinetics of delta-13CO2 using laser spectroscopy. *J Gastroenterol Hepatol* 1998; **13**: 732-737
79 **Gisbert JP**, Benito LM, Lara S, Vazquez A, Jimenez I, Pajares JM. 13C-urea breath test for the diagnosis of Helicobacter pylori infection: are basal samples necessary? *Eur J Gastroenterol Hepatol* 2000; **12**: 1201-1205
80 **Suto H**, Azuma T, Ito S, Ito Y, Miyaji H, Yamazaki Y, Kohli Y, Kuriyama M. Endoscopic [13C]-urea breath test for quantification of Helicobacter pylori infection. *J Gastroenterol Hepatol* 2000; **15**: 161-167
81 **Kubota K**, Shimoyama S, Shimizu N, Noguchi C, Mafune K, Kaminishi M, Tange T. Studies of 13C-urea breath test for diagnosis of Helicobacter pylori infection in patients after partial gastrectomy. *Digestion* 2002; **65**: 82-86
82 **Kato M**, Saito M, Fukuda S, Kato C, Ohara S, Hamada S, Nagashima R, Obara K, Suzuki M, Honda H, Asaka M, Toyota T. 13C-Urea breath test, using a new compact nondispersive isotope-selective infrared spectrophotometer: comparison with mass spectrometry. *J Gastroenterol* 2004; **39**: 629-634
83 **Gisbert JP**, Olivares D, Jimenez I, Pajares JM. Long-term follow-up of 13C-urea breath test results after Helicobacter pylori eradication: frequency and significance of borderline delta13CO2 values. *Aliment Pharmacol Ther* 2006; **23**: 275-280
84 **Lotterer E**, Ramaker J, Ludtke FE, Tegeler R, Geletneky JV, Bauer FE. The simplified 13C-urea breath test--one point analysis for detection of Helicobacter pylori infection. *Z Gastroenterol* 1991; **29**: 590-594
85 **Lotterer E**, Ludtke FE, Tegeler R, Lepsien G, Bauer FE. The 13C-urea breath test--detection of Helicobacter pylori infection in patients with partial gastrectomy. *Z Gastroenterol* 1993; **31**: 115-119
86 **Labenz J**, Barsch G, Peitz U, Aygen S, Hennemann O, Tillenburg B, Becker T, Stolte M. Validity of a novel biopsy urease test (HUT) and a simplified 13C-urea breath test for diagnosis of Helicobacter pylori infection and estimation of the severity of gastritis. *Digestion* 1996; **57**: 391-397
87 **Ellenrieder V**, Glasbrenner B, Stoffels C, Weiler S, Bode G, Moller P, Adler G. Qualitative and semi-quantitative value of a modified 13C-urea breath test for identification of Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 1997; **9**: 1085-1089
88 **Leodolter A**, Dominguez-Munoz JE, von Arnim U, Manes G, Malfertheiner P. 13C-urea breath test for the diagnosis of Helicobacter pylori infection. A further simplification for clinical practice. *Scand J Gastroenterol* 1998; **33**: 267-270
89 **Perri F**, Clemente R, Pastore M, Quitadamo M, Festa V, Bisceglia M, Li Bergoli M, Lauriola G, Leandro G, Ghoos Y, Rutgeerts P, Andriulli A. The 13C-urea breath test as a predictor of intragastric bacterial load and severity of Helicobacter pylori gastritis. *Scand J Clin Lab Invest* 1998; **58**: 19-27
90 **Coelho LG**, Reber M, Passos MC, Aguiar RO, Casaes PE, Bueno ML, Yazaki FR, Castro FJ, Vieira WL, Franco JM, Castro LP. Application of isotope-selective non-dispersive infrared spectrometry for the evaluation of the 13C-urea breath test: comparison with three concordant methods. *Braz J Med Biol Res* 1999; **32**: 1493-1497
91 **Mock T**, Yatscoff R, Foster R, Hyun JH, Chung IS, Shim CS, Yacyshyn B. Clinical validation of the Helikit: a 13C urea breath test used for the diagnosis of Helicobacter pylori infection. *Clin Biochem* 1999; **32**: 59-63
92 **Mana F**, Franken PR, Ham HR, Reynaert H, Urbain D. 13C urea breath test with nondispersive isotope-selective infrared spectrometry: reproducibility and importance of the fasting status. *Helicobacter* 2000; **5**: 104-108
93 **Niv Y**, Niv G, Koren R. 13C-urea breath test for diagnosis of Helicobacter pylori infection in the elderly. *Dig Dis Sci* 2004; **49**: 1840-1844
94 **Parente F**, Bianchi Porro G. The (13)C-urea breath test for non-invasive diagnosis of Helicobacter pylori infection: which procedure and which measuring equipment? *Eur J Gastroenterol Hepatol* 2001; **13**: 803-806
95 **Gatta L**, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, Miglioli M, Vaira D. Effect of proton pump inhibitors and antacid therapy on 13C urea breath tests and stool test for Helicobacter pylori infection. *Am J Gastroenterol* 2004; **99**: 823-829
96 **Bielanski W**, Konturek SJ. New approach to 13C-urea breath test: capsule-based modification with low-dose of 13C-urea in the diagnosis of Helicobacter pylori infection. *J Physiol Pharmacol* 1996; **47**: 545-553
97 **Gisbert JP**, Gomollon F, Dominguez-Munoz JE, Borda F, Jimenez I, Vazquez MA, Gallego S, Iglesias J, Pastor G, Pajares JM. Comparison between two 13C-urea breath tests for the diagnosis of Helicobacter pylori infection: isotope ratio mass spectrometer versus infrared spectrometer. *Gastroenterol Hepatol* 2003; **26**: 141-146
98 **Graham DY**, Runke D, Anderson SY, Malaty HM, Klein PD. Citric acid as the test meal for the 13C-urea breath test. *Am J Gastroenterol* 1999; **94**: 1214-1217
99 **Yong CS**, Kim YI, Park SM, Kwon R, Han HH, Park JG, Yang CY, Kim JA, Yoo BK, Rhee JD, Choi HG. Trials of novel 13C-urea-containing capsule for more economic and sensitive diagnosis of Helicobacter pylori infection in human subjects. *Arch Pharm Res* 2006; **29**: 879-883
100 **Pantoflickova D**, Scott DR, Sachs G, Dorta G, Blum AL. 13C urea breath test (UBT) in the diagnosis of Helicobacter pylori: why does it work better with acid test meals? *Gut* 2003; **52**: 933-937
101 **Wang WM**, Lee SC, Ding HJ, Jan CM, Chen LT, Wu DC, Liu CS, Peng CF, Chen YW, Huang YF, Chen CY. Quantification of Helicobacter pylori infection: Simple and rapid 13C-urea breath test in Taiwan. *J Gastroenterol* 1998; **33**: 330-335
102 **Wang WM**, Lee SC, Wu DC, Chen LT, Liu CS, Peng CF, Ding HJ, Chen CY, Jan CM. Simplified 13C-urea breath test for the diagnosis of Helicobacter pylori infection--the availability of without fasting and without test meal. *Kaohsiung J Med Sci* 2000; **16**: 607-613
103 **Casellas F**, Lopez J, Borruel N, Saperas E, Vergara M, de Torres I, Armengol JR, Malagelada JR. The impact of delaying gastric emptying by either meal substrate or drug on the

[13C]-urea breath test. *Am J Gastroenterol* 1999; **94**: 369-373
104 **Oksanen A**, Bergstrom M, Sjostedt S, Gad A, Hammarlund B, Seensalu R. Accurate detection of Helicobacter pylori infection with a simplified 13C urea breath test. *Scand J Clin Lab Invest* 1997; **57**: 689-694
105 **Pytko-Polonczyk J**, Konturek SJ, Karczewska E, Bielanski W, Kaczmarczyk-Stachowska A. Oral cavity as permanent reservoir of Helicobacter pylori and potential source of reinfection. *J Physiol Pharmacol* 1996; **47**: 121-129
106 **Hillman JD**, Socransky SS, Shivers M. The relationships between streptococcal species and periodontopathic bacteria in human dental plaque. *Arch Oral Biol* 1985; **30**: 791-795
107 **Winiarski M**, Bielanski W, Plonka M, Dobrzanska M, Kaminska A, Bobrzynski A, Ronturek PC, Konturek SJ. The usefulness of capsulated 13C-urea breath test in diagnosis of Helicobacter pylori infection in patients with upper gastrointestinal bleeding. *J Clin Gastroenterol* 2003; **37**: 34-38
108 **Isomoto H**, Inoue K, Shikuwa S, Furusu H, Nishiyama T, Omagari K, Mizuta Y, Murase K, Murata I, Enjoji A, Kanematsu T, Kohno S. Five minute endoscopic urea breath test with 25 mg of (13)C-urea in the management of Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 2002; **14**: 1093-1100
109 **Peng NJ**, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, Tseng HH, Huang WK, Lo GH, Hsu PI. Endoscopic 13C-urea breath test for the diagnosis of Helicobacter pylori infection. *Dig Liver Dis* 2003; **35**: 73-77
110 **Lee TH**, Yang JC, Lee SC, Farn SS, Wang TH. Effect of mouth washing on the. *J Gastroenterol Hepatol* 2001; **16**: 261-263
111 **Ormand JA**, Talley NJ. Campylobacter pylori, mucus, and peptic ulceration. A dynamic interaction. *J Clin Gastroenterol* 1989; **11**: 492-495
112 **Miederer SE**, Grubel P. Profound increase of Helicobacter pylori urease activity in gastric antral mucosa at low pH. *Dig Dis Sci* 1996; **41**: 944-949
113 **Atherton JC**, Washington N, Blackshaw PE, Greaves JL, Perkins AC, Hawkey CJ, Spiller RC. Effect of a test meal on the intragastric distribution of urea in the 13C-urea breath test for Helicobacter pylori. *Gut* 1995; **36**: 337-340

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RAPID COMMUNICATION

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

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Abstract

AIM: To investigate the poor prognosis of HCC with PVTT, we evaluated the efficacy by a new combination chemotherapy for advanced hepatocellular carcinoma (HCC) with portal vein tumor thrombus (PVTT).

METHODS: From 2002 to 2007, a total of 10 consecutive patients with Stage IVA HCC accompanied by PVTT were studied prospectively to examine the efficacy of treatment by intra-arterial infusion of a chemotherapeutic agents consisting of etoposide, carboplatin, epirubicin and pharmacokinetic modulating chemotherapy by 5-FU and enteric-coated tegafur/uracil.

RESULTS: The mean course of chemotherapy was 14.4 (range, 9-21) mo. One patient showed complete response (CR) with disappearance of HCC and PVTT after treatment, and the two patients showed partial response (PR), response rate (CR + PR/All cases 30%). The median survival time after the therapy was 457.2 d. The one-year survival rate was 70%. Adverse reactions were tolerable.

CONCLUSION: Although the prognosis of most patients with Stage IVA HCC by PVTT is poor, our combination chemotherapy may induces long-term survival and is an effective treatment and produced anti-tumor activity with tolerable adverse effects in patients for advanced Stage IVA HCC accompanied by PVTT.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide including Japan[1]. Although the development of imaging modalities has made the early diagnosis of HCC possible, surgically resectable cases are relatively uncommon because of a hepatic function reserve and/or an advanced stage at presentation. Several modalities, such as percutaneous ethanol injection (PEI), transcatheter arterial embolization (TAE), chemolipiodolization, microwave coagulation therapy (MCT), and radiofrequency ablation therapy (RFA) are reportedly useful in treating patients with unresectable disease[2,3]. However, unfortunately, many hepatocellular carcinoma patients have tumor recurrence. Furthermore, HCC has a predilection for portal vein invasion, which has been shown to be a poor prognostic factor. An effective therapy regimen is needed for advanced HCC with portal vein tumor thrombus (PVTT). Recent trials have been reported that combination therapy of intra-arterial 5-FU and systemic interferon for HCC with PVTT is effective[4,5]. However, portal venous invasion is a crucial factor that can worsen the prognosis of patients with HCC. It often leads to extensive spreading of the tumor throughout the liver, and can increase portal venous blood pressure, resulting in the fatal rupture of esophageal varices, and can decrease portal flow which causes ascites, jaundice, hepatic encephalopathy, and liver failure. Previous studies have

reported that the median survival time of patients with portal venous invasion was 2.7-4 mo if left untreated[6,7].

As systemic therapy of HCC, we had previously achieved complete remission of multiple HCC associated with hepatitis C virus-related decompensated liver cirrhosis by oral administration of enteric-coated tegafur/uracil[8]. Furthermore, we reported that oral administration of enteric-coated tegafur/uracil induces long-term survival and is an effective treatment for Stage Ⅳ-A HCC[9]. However, this therapy by single agent is not effective HCC with PVTT. Therefore, there is an urgent need for new method and active drugs for PVTT from HCC.

The aim of this study is to evaluate the usefulness by intra-arterial infusion of a chemotherapeutic agents consisting of etoposide, carboplatin, epirubicin and pharmacokinetic modulating chemotherapy by 5-FU and enteric-coated tegafur/uracil for HCC with tumor thrombosis of the main trunk of the portal vein.

MATERIALS AND METHODS

Ethics

The study protocol was reviewed and approved by the Hospital Ethics Committee. Informed consent was obtained from each patient who entered a randomized controlled trial and from family member(s).

Patients

A group of 10 consecutive patients with HCC accompanied by portal vein tumor thrombus (PVTT) were enrolled in the therapeutic trial between April 2002 and April 2007. The diagnosis of HCC was made by histologically and/or imaging study.

All patients received intra-arterial regional chemotherapy carried out at the Department of Gastroenterology and Hepatology, Saiseikai Niigata Second Hospital and gave their informed consent according to our situational guidelines, and the study received ethical approval.

Eligibility criteria for patients in this study included the following: (1) diagnosed Stage ⅣA HCC with PVTT (2) unresectable carefully assessed by the individual experts; (3) no recent active treatments including surgery, radiotherapy, chemotherapy, transarterial embolization, percutaneous ethanol injection, or other regional treatment within six month; (4) HCC with PVTT diagnosed by total image systems such as computed tomography (CT) or magnetic resonance imaging (MRI). (5) the ability to manage the indwelling catheters and implanted injection ports; (6) adequate hematologic function (white blood cell count > 3000/L, platelet count > 80 000/L, and hemoglobin level > 9.5 gm/dL); adequate renal function (serum creatinine < 1.5 mg/dL and a creatinine clearance > 60 mL/min); (7) adequate hepatic function, (8) a performance status less than 3 at pre-treatment, (9) portal tumor thrombi located the first portal branch, or the main portal trunk, and (10) informed consent.

Treatment schedule and follow up

For intra-arterial regional chemotherapy, catheters were introduced into the proper or common hepatic artery placed *via* the right femoral artery using the Seldinger method. The gastroduodenal artery and the right artery were occluded by a steel coil as indicated to prevent gastroduodenal injury from the anticancer agents. Intra-arterial regional chemotherapy was performed by puncturing a thin needle percutaneously into the port. Before every infusion, we confirmed that the catheters were patent, either by checking the blood back-flowing in the catheter or by injecting a contrast medium under fluoroscopy. Using an infusion pump (Syringe Pump, Terumo Co. Ltd., Osaka, Japan), 50 mg/body of etoposide (VePesid, Bristol-Myers Squibb Co. Ltd., Tokyo, Japan), 300 mg/body of carboplatin (Paraplatin, Bristol-Myers Squibb Co. Ltd., Tokyo, Japan) and 60 mg/body of epirubicin (Farmorubicin, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) were infused from each catheter over a 30-minute period. Subsequently, a continuous arterial infusion of 5-FU (500 mg/m^2) (5-FU, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) was given for 24 h. Before the needle was removed from the port, both the port and connected catheter were filled with undiluted heparin (1000 U/mL). An antiemetic and an H2-receptor antagonist were given intravenously. This treatment was repeated once weekly for 3 consecutive weeks of every 4 wk or biweekly, mainly at our outpatient clinic, for as long as possible. All patients were given also enteric-coated tegafur/uracil (Taiho Pharmaceutical, Co. Ltd., Tokyo, Japan) at a dose rate of 200 mg/body twice daily. Treatment was continued for a minimum of 8 wk and given until patient withdrawal or death even after the progression of disease.

Studies during follow-up included a physical examination, complete blood count, platelet count, and determination of levels of AFP, PIVKA-Ⅱ, amylase, liver transaminase, urea nitrogen, and creatinine. Either an ultrasonogram or a computed tomogram of the abdomen was obtained at least every two months, to determine the size of HCC and PVTT.

Evaluation of therapeutic response

The therapeutic clinical response of the liver was assessed in accordance with the World Health Organization Criteria. Clinical responses were graded as follows. Complete response (CR) was defined as disappearance of all measurable lesions in the liver, continuing for at least 4 weeks when assessed by both computed tomography and ultrasonography. Reduction of tumor size by more than 50%, continuing for at least 4 wk, was regarded as partial response (PR). No change (NC) was determined as tumors showing a decrease in size of less than 25%. Progressive disease (PD) was defined as tumors that had grown over 25%.

Statistical analysis

Survival curves were calculated by the Kaplan-Meier method and the difference between survival curves was evaluated using the log-rank test.

Statistical analyses were performed using Stat View-J4.11 software (Abacus Concepts; Berkeley, California) to assess the relative prognostic importance

Table 1 Characteristics of the patients

10 patients
Age mean 60.20 y.o (range 35-70)
Sex: Male/Female 7/3
Child-Pugh's stage: A,B/C 8/2
"Virus: HBsAg/anti-HCV 8/2"

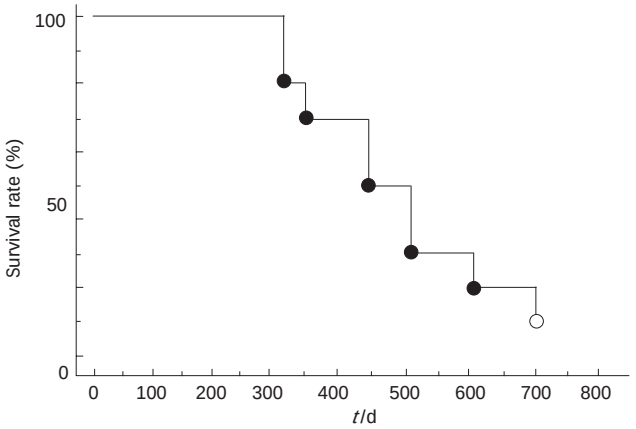

Figure 1 Cumulative survival of patients with hepatocellular carcinoma accompanied by PVTT who treated combination therapy.

of variables in predicting the survival rate. Differences at $P < 0.05$ were considered significant.

RESULTS

Background clinical and laboratory data of patients

Patients' profiles before the combination chemotherapy are listed in Table 1. Seven men and three women, with a median age of 60.2 years, were treated. Positive HBsAg was found in 70% of patients, while anti-hepatitis C virus (HCV) serology was positive in 30% of patients.

Child-Pugh Grade A/B was 2/6, the remaining patients had Grade C. Only 2 patients received the full courses of chemotherapy. The median number of cycles received per patients was 15.3. Dose reduction was required in 80% of the patients, mainly due to profound bone marrow suppression from the previous cycle.

Cumulative survival rate of patients by Kaplan-Meier survival curves

Of the 10 patients in the treated group, a total of 3 (30.0%) were classed as CR or PR, and a total 7 (70.0%) were classed as NC or PD. At the time of the final analysis (April 2007) 2 patients (from the treatment group) were alive. A total of 153 treatment cycles were administered.

The overall survival curve for all 10 patients is shown in Figure 1. The median survival was 457.2 d. The survival rates at the end of 1-year and 2-year were 70.0% and 20.0%, respectively. Of the 10 patients studied, 8 had died by the time of this analysis. The survival curves for clinical responders (CR or PR) and the others (NC or PD) are shown in Figure 2. The 6-mo and one-year survival rates were 100% and 100.0%, respectively, in the responders

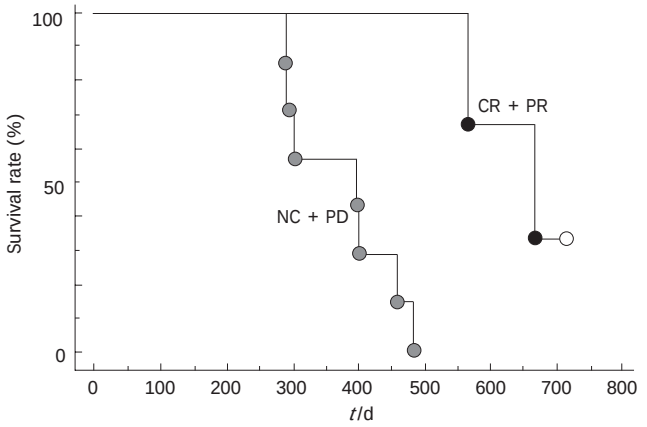

Figure 2 Survival of patients with hepatocellular carcinoma accompanied by PVTT according to the response and control (*Log-rank P < 0.05)*. CR: Complete response, PR**;** Partial response, NC; No change, PD; Progressive disease.

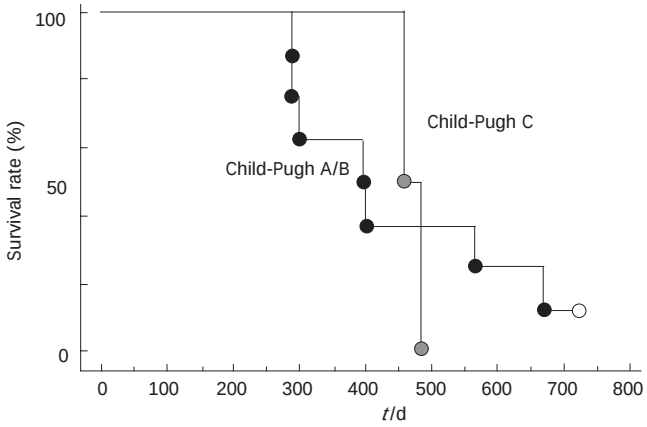

Figure 3 Survival of patients with hepatocellular carcinoma accompanied by PVTT according to Child-Pugh's stage (*Log-rank P = 0.98)*.

and 100% and 57.1%, respectively, in the non-responder. There was a significant difference in survival between the two groups ($P < 0.01$).

However, there was not a significant difference in survival rates between Child-Pugh A/B group and Child-Pugh C group (Figure 3).

Side effects and complications due to regional chemotherapy

The side-effects and complications encountered during therapy are summarized in Table 2. Local complications at the femoral artery entry sites did not occur in any patient. No serious complications that necessitated intensive care were encountered during therapy. The side-effects included oral dryness, diarrhea and liver dysfunction, and bone marrow suppression. Mild oral dryness was noted at the beginning of the treatment in 40.0% of patients, but this subsided as treatment continued. Mild diarrhea was noted at the beginning of the treatment in 30.0% of patients, but this also subsided with time. Such symptoms resolved spontaneously or after appropriate therapy. The complications experienced by patients treated with regional chemotherapy were well tolerated. No other serious

Table 2 Main clinical side effects observed during the treatment (*n* represents the number of patients having experienceed the effect in any of the courses)

	Grade of toxicity			
	1	2	3	4
Oral dryness	3	1	0	0
Diarrhea	1	2	0	0
Vomiting	1	0	0	0
Liver dysfunction	1	2	0	0
Fever	0	0	0	0
Hair loss	1	0	0	0
BM suppression	4	4	0	0

complications, such as gastric ulcer, liver damage, renal damage, vascular complications, or cardiac toxicity were encountered. However, dose reduction was required in 80% of the patients, mainly due to profound bone marrow suppression from the previous cycle. There were no treatment-related deaths from administration of regional chemotherapy.

DISCUSSION

Patients with HCC are highly compromised by failing liver function. HCC is associated with a high risk of portal vein involvement. PVTT is one of important prognostic factors in patients with HCC. However, HCC with PVTT is refractory to treatment. The treatment of HCC with PVTT is still problematic and major challenge item for oncologists because of its high and dismal outcomes. None of the reported treatment regimens can be considered to be standard treatment for HCC with PVTT.

Meanwhile, regional hepatic arterial infusion chemotherapy is a reasonable drug delivery system for patients with advanced HCC because the tumors derive most of their blood supply from the hepatic artery, whereas the portal vein supplies the normal parenchyma[10]. Furthermore, chemotherapy combined with interferon is reported to be effective for HCC with PVTT.

Combined treatment with 5-FU and alpha-interferon for HCC patients was first reported by Patt, *et al* in 1993[11]. The response rate was reportedly 22%. Urabe *et al*[12] treated 16 patients with HCC and PVTT in the main trunk or the major branches of the portal vein by intrahepatic infusion of methotrexate, 5-FU, and cisplatin, and administered alpha-interferon subcutaneously. The response rate and median survival time were 46.7% and 7 mo, respectively.

Moreover, combined intra-arterial 5-FU and subcutaneous alpha-interferon therapy for 8 patients with HCC accompanied by PVTT in the major portal vein was reported by Sakon *et al*[5]. The response rate was 63%. In another study by this group in 2005, 55 patients received this treatment, and 8 (14.5%) showed a complete response, 16 (29.1%) showed a partial response, 4 (7.3%) showed no response, and 27 (49.1%) showed progressive disease[13]. The median survival time and 5-year survival rate were 11.8 mo and 16.4%, respectively. Using this combination protocol, Obi *et al*[4] treated 116 patients with unresectable HCC accompanied by PVTT in the main trunk or the 1st branches of the portal vein. The survival rates at 1 and 2 years among overall patients were 34% and 18%, respectively, in contrast to 15% and 5% among the historical controls. Survival rates at 1 and 2 years were 81% and 59% among complete responders, respectively, and 43% and 18% among partial responders. The median survival time was prolonged to 11 mo in patients with an active response, although there appears to be no benefit in patients without an active response.

However, it is chaotic to determine whether the combination chemotherapy with interferon is effective or not for HCC accompanied by PVTT.

We previously reported[8,9] that administration of enteric-coated tegafur/uracil induces long-term survival and is an effective treatment for Stage Ⅳ-A HCC. However, single agent such as enteric-coated tegafur/uracil was not effective for HCC with PVTT. So, combination chemotherapy is needed for HCC with PVTT.

The choice of the anticancer agent is important in achieving favorable clinical results. We selected etoposide, carboplatin, epirubicin and pharmacokinetic modulating chemotherapy by 5-FU and enteric-coated tegafur/uracil.

Firstly, etoposide is agent which has shown significant antitumor against HCC[14-16]. It could be suggested as part of intensive multidrug regimens for HCC and high-risk HBV[17-19]. Though response rates to cisplatin and etoposide[20] given systemically as single agents are 5 and 15%, intra-arterial combination chemotherapy using cisplatin and etoposide produces a high rate of objective tumor remissions in patients with HCC[21].

However, cisplatin has been reported that it has a lot of nephrotoxic and emetic effects. To the contrary, carboplatin has demonstrated antitumor activity comparable to cisplatin and has been shown to have fewer nephrotoxic and emetic effects. In fact, carboplatin is thought to be a useful anticancer agent in patients with HCC treated with TACE. Furthermore, it is reported that carboplatin is effective for HCC[22-24].

So we selected carboplatin as combination with etoposide. In addition, a combination of epirubicin and etoposide appears to be an active and tolerable therapeutic option for HCC patients who are not candidates for surgical or locoregional procedures[19,25-27].

Recently, Kusunoki *et al* reported that pharmacokinetic modulating chemotherapy, based on the concept that the benefit of a continuous venous 5-fluorouracil infusion can be potentiated by low-dose oral tegafur/uracil is useful for a variety of cancers[28,29].

In fact, it is reported that modified pharmacokinetic modulating chemotherapy had no severe side effect and was effective for advanced unresectable HCC[30].

Based on these facts, we tried combination chemotherapy for HCC with PVTT. In our series, the treatment resulted in an objective response rate of 30% and a median survival of 457.2 d. Only the three patients who had an objective response had a survival of long duration.

As our group was small, we did not perform a statistical analysis to determine a predictive factor for response. However, our results are comparable with those of most interferon combination chemotherapy.

In our study, the toxicity of this therapy was low despite the fact that all of the patients had cirrhosis. It is noteworthy that there were no patients showing overt liver toxicity. Moreover, there was no treatment-related death.

In this study, no hepatotoxicity due to this combination chemotherapy was observed. The side effects of this regimen were minimal and well tolerated.

In conclusion, this chemotherapeutic regimen ameliorated the survival of patients with advanced HCC without serious adverse effects.

We suggest that, in the near future, this chemotherapy method should be subjected to a prospective randomized controlled study for HCC with PVTT. Further prospective randomized clinical trials of chemotherapy for HCC with PVTT will be needed.

COMMENTS

Background

Portal venous tumor thrombus (PVTT) is a crucial factor that can worsen the prognosis of patients with hepatocellular carcinoma (HCC). It often leads to extensive spreading of the tumor throughout the liver, and can increase portal venous blood pressure, resulting in the fatal rupture of esophageal varices, and can decrease portal flow which causes ascites, jaundice, hepatic encephalopathy, and liver failure. However, there is not effective useful therapy for HCC combined with PVTT. Therefore, there is an urgent need for new and active drugs and combination chemotherapy of advanced HCC.

Research frontiers

HCC has a predilection for portal vein invasion, which has been shown to be a poor prognostic factor. An effective therapy regimen is needed for advanced HCC with PVTT. Combination chemotherapy is needed for HCC with PVTT urgently. The choice of the anticancer agent is important in achieving favorable clinical results. The authors selected etoposide, carboplatin, epirubicin and pharmacokinetic modulating chemotherapy by 5-FU and enteric-coated tegafur/uracil. Progress in implantable drug delivery systems has made possible the repeated arterial infusion of chemotherapeutic agents for patients with advanced HCC recently. Therefore, hepatic arterial infusion chemotherapy has been often selected as a therapeutic option for advanced HCC with PVTT.

Innovations and breakthroughs

The authors investigated the efficacy, the feasibility, usefulness, and complication rate of arterial combination therapies for HCC with PVTT.

Applications

Intra-arterial combination chemotherapy is useful and inducing long-term survival for advanced HCC accompanied by PVTT. Further prospective randomized clinical trials of chemotherapy for HCC with PVTT will be needed.

Terminology

PVTT: Portal vein tumor thrombus meaning tumor thrombus locating the first portal branch, or the main portal trunk.

Peer review

This is an interesting manuscript reporting the strategy for HCC with PVTT. This new information is certainly worthy of publication.

REFERENCES

1 **Okuda K**. Hepatocellular carcinoma: recent progress. *Hepatology* 1992; **15**: 948-963

2 **Ichida T**, Van Thiel DH, Hassanein T. The medical management of hepatocellular carcinoma (HCC) in Japan: a review with implications for HCC seen in the west. *Hepatogastroenterology* 1996; **43**: 1575-1583

3 **Livraghi T**, Lazzaroni S, Meloni F. Radiofrequency thermal ablation of hepatocellular carcinoma. *Eur J Ultrasound* 2001; **13**: 159-166

4 **Obi S**, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, Tateishi R, Teratani T, Shiina S, Omata M. Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2006; **106**: 1990-1997

5 **Sakon M**, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, Kawata S, Imai Y, Iijima S, Monden M. Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* 2002; **94**: 435-442

6 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Bru C, Rodes J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67

7 **Villa E**, Moles A, Ferretti I, Buttafoco P, Grottola A, Del Buono M, De Santis M, Manenti F. Natural history of inoperable hepatocellular carcinoma: estrogen receptors' status in the tumor is the strongest prognostic factor for survival. *Hepatology* 2000; **32**: 233-238

8 **Ishikawa T**, Ichida T, Ishimoto Y, Yokoyama J, Nomoto M, Ebe Y, Usuda H, Naito M, Asakura H. Complete remission of multiple hepatocellular carcinomas associated with hepatitis C virus-related, decompensated liver cirrhosis by oral administration of enteric-coated tegafur/uracil. *Am J Gastroenterol* 1999; **94**: 1682-1685

9 **Ishikawa T**, Ichida T, Sugitani S, Tsuboi Y, Genda T, Sugahara S, Uehara K, Inayoshi J, Yokoyama J, Ishimoto Y, Asakura H. Improved survival with oral administration of enteric-coated tegafur/uracil for advanced stage IV-A hepatocellular carcinoma. *J Gastroenterol Hepatol* 2001; **16**: 452-459

10 **Breedis C**, Young G. The blood supply of neoplasms in the liver. *Am J Pathol* 1954; **30**: 969-977

11 **Patt YZ**, Yoffe B, Charnsangavej C, Pazdur R, Fischer H, Cleary K, Roh M, Smith R, Noonan CA, Levin B. Low serum alpha-fetoprotein level in patients with hepatocellular carcinoma as a predictor of response to 5-FU and interferon-alpha-2b. *Cancer* 1993; **72**: 2574-2582

12 **Urabe T**, Kaneko S, Matsushita E, Unoura M, Kobayashi K. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorouracil, cisplatin and subcutaneous interferon-alpha-2b for patients with locally advanced hepatocellular carcinoma. *Oncology* 1998; **55**: 39-47

13 **Ota H**, Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, Nakamura M, Damdinsuren B, Wada H, Marubashi S, Miyamoto A, Dono K, Umeshita K, Nakamori S, Wakasa K, Monden M. Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaneous interferon-alpha and intra-arterial 5-fluorouracil; role of type 1 interferon receptor expression. *Br J Cancer* 2005; **93**: 557-564

14 **Yodono H**, Sasaki T, Tarusawa K, Midorikawa H, Saito Y, Takekawa SD. Arterial infusion chemotherapy for advanced hepatocellular carcinoma using EPF and EAP therapies. *Cancer Chemother Pharmacol* 1992; **31** Suppl: S89-S92

15 **Yodono H**, Takekawa SD, Tarusawa K, Ikami I, Kanehira J, Saito Y, Takahashi S, Sasaki T, Nishi N, Kimura T. Combination therapy consisting of arterial infusion chemotherapy (EPF, EAP) and transcatheter arterial embolization (TAE). *Cancer Chemother Pharmacol* 1994; **33** Suppl: S79-S83

16 **Wierzbicki R**, Ezzat A, Abdel-Warith A, Ayoub A, Kagevi I, Fadda M, Sieck J, Abdulkareem M, Amin T, Yazigi A. Phase II trial of chronic daily VP-16 administration in unresectable hepatocellular carcinoma (HCC). *Ann Oncol* 1994; **5**: 466-467

17 **Casanova M**, Massimino M, Ferrari A, Spreafico F, Piva L, Coppa J, Luksch R, Cefalo G, Terenziani M, Polastri D, Bellani FF, Mazzaferro V. Etoposide, cisplatin, epirubicin chemotherapy in the treatment of pediatric liver tumors. *Pediatr Hematol Oncol* 2005; **22**: 189-198

18 **Aita P**, Robieux I, Sorio R, Tumolo S, Corona G, Cannizzaro R, Colussi AM, Boiocchi M, Toffoli G. Pharmacokinetics of oral etoposide in patients with hepatocellular carcinoma. *Cancer*

Chemother Pharmacol 1999; **43**: 287-294
19 **Bobbio-Pallavicini E**, Porta C, Moroni M, Bertulezzi G, Civelli L, Pugliese P, Nastasi G. Epirubicin and etoposide combination chemotherapy to treat hepatocellular carcinoma patients: a phase II study. *Eur J Cancer* 1997; **33**: 1784-1788
20 **Melia WM**, Johnson PJ, Williams R. Induction of remission in hepatocellular carcinoma. A comparison of VP 16 with adriamycin. *Cancer* 1983; **51**: 206-210
21 **Sangro B**, Rios R, Bilbao I, Beloqui O, Herrero JI, Quiroga J, Prieto J. Efficacy and toxicity of intra-arterial cisplatin and etoposide for advanced hepatocellular carcinoma. *Oncology* 2002; **62**: 293-298
22 **Akimoto M**, Yoshikawa M, Ebara M, Sato T, Fukuda H, Kondo F, Saisho H. Relationship between therapeutic efficacy of arterial infusion chemotherapy and expression of P-glycoprotein and p53 protein in advanced hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 868-873
23 **Yamashita F**, Tanaka M, Andou E, Yutani S, Kato O, Tanikawa K. Carboplatin as an anticancer agent for transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *Oncology* 1997; **54**: 28-33
24 **Kim SJ**, Seo HY, Choi JG, Sul HR, Sung HJ, Park KH, Choi IK, Oh SC, Yoon SY, Seo JH, Choi CW, Kim BS, Shin SW, Kim YH, Kim JS. Phase II study with a combination of epirubicin, cisplatin, UFT, and leucovorin in advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2006; **57**: 436-442
25 **Boucher E**, Corbinais S, Brissot P, Boudjema K, Raoul JL. Treatment of hepatocellular carcinoma (HCC) with systemic chemotherapy combining epirubicin, cisplatinum and infusional 5-fluorouracil (ECF regimen). *Cancer Chemother Pharmacol* 2002; **50**: 305-308
26 **Pohl J**, Zuna I, Stremmel W, Rudi J. Systemic chemotherapy with epirubicin for treatment of advanced or multifocal hepatocellular carcinoma. *Chemotherapy* 2001; **47**: 359-365
27 **Dobbs NA**, Twelves CJ, Rizzi P, Warwick JD, Metivier EM, Williams R, Johnson PJ. Epirubicin in hepatocellular carcinoma: pharmacokinetics and clinical activity. *Cancer Chemother Pharmacol* 1994; **34**: 405-410
28 **Kusunoki M**, Yanagi H, Kotera H, Noda M, Yamamura T. Effects of pharmacokinetic modulating chemotherapy using oral UFT and continuous venous 5FU infusion on the prognosis of irradiated rectal carcinomas with p53 overexpression. *Int J Oncol* 1998; **13**: 653-657
29 **Kusunoki M**, Yanagi H, Noda M, Yoshikawa R, Yamamura T. Results of pharmacokinetic modulating chemotherapy in combination with hepatic arterial 5-fluorouracil infusion and oral UFT after resection of hepatic colorectal metastases. *Cancer* 2000; **89**: 1228-1235
30 **Kamiyama T**, Matsushita M, Kurauchi N, Nakagawa T, Kamachi H, Kondo M, Ito T, Ogata T, Nishikawa M, Todo S. Modified pharmacokinetic modulation chemotherapy (PMC) with medication of UFT and intraarterial infusion of 5-FU for advanced unresectable HCC. *Gan To Kagaku Ryoho* 2002; **29**: 2527-2531

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RAPID COMMUNICATION

Pre- and postoperative systemic hemodynamic evaluation in patients subjected to esophagogastric devascularization plus splenectomy and distal splenorenal shunt: A comparative study in schistomomal portal hypertension

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Abstract

AIM: To investigate the systemic hemodynamic effects of two surgical procedures largely employed for treatment of schistosomal portal hypertension.

METHODS: Thirty-six patients undergoing elective surgical treatment of portal hypertension due to hepatosplenic mansonic schistosomiasis were prospectively evaluated. All patients were subjected to preoperative pulmonary artery catheterization; 17 were submitted to esophagogastric devascularization and splenectomy (EGDS) and 19 to distal splenorenal shunt (DSRS). The systemic hemodynamic assessment was repeated 4 d after the surgical procedure.

RESULTS: Preoperative evaluation revealed (mean ± SD) an increased cardiac index (4.78 ± 1.13 L/min per m^2), associated with a reduction in systemic vascular resistance index (1457 ± 380.7 dynes.s/cm^5.m^2). The mean pulmonary artery pressure (18 ± 5.1 mmHg) as well as the right atrial pressure (7.9 ± 2.5 mmHg) were increased, while the pulmonary vascular resistance index (133 ± 62 dynes.s/cm^5.m^2) was decreased. Four days after EGDS, a significant reduction in cardiac index (3.80 ± 0.4 L/min per m^2, $P < 0.001$) and increase in systemic vascular resistance index (1901.4 ± 330.2 dynes.s/cm^5.m^2, $P < 0.001$) toward normal levels were observed. There was also a significant reduction in pulmonary artery pressure (12.65 ± 4.7 mmHg, $P < 0.001$) and no significant changes in the pulmonary vascular resistance index (141.6 ± 102.9 dynes.s/cm^5.m^2). Four days after DSRS, a non-significant increase in cardiac index (5.2 ± 0.76 L/min per m^2) and systemic vascular resistance index (1389 ± 311 dynes.s/cm^5.m^2) was observed. There was also a non-significant increase in pulmonary artery pressure (19.84 ± 5.2 mmHg), right cardiac work index (1.38 ± 0.4 kg.m/m^2) and right ventricular systolic work index (16.3 ± 6.3 g.m/m^2), without significant changes in the pulmonary vascular resistance index (139.7 ± 67.8 dynes.s/cm^5.m^2).

CONCLUSION: The hyperdynamic circulatory state observed in mansonic schistosomiasis was corrected by EGDS, but was maintained in patients who underwent DSRS. Similarly, the elevated mean pulmonary artery pressure was corrected after EGDS and maintained after DSRS. EGDS seems to be the most physiologic surgery for patients with schistosomal portal hypertension.

Key words: Pulmonary Hypertension; Hyperdynamic circulation; Portal Hypertension; Splenectomy; Cardiomyopathy

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INTRODUCTION

In Brazil, mansonic schistosomiasis is an endemic disease, and its hepatosplenic form, which is characterized by presinusoidal portal hypertension with preserved liver function and marked splenomegaly, is a major cause of portal hypertension[1-3]. Upper digestive tract hemorrhage due to esophageal varices rupture is the most feared complication[4].

The development of portal hypertension, regardless of its etiology, is a consequence of increased vascular resistance, mostly due to an architectural distortion of

the liver parenchyma secondary to fibrosis, but also due to a diminished endothelial nitric oxide release from the hepatic endothelium[5]. Increased portal venous inflow due to mesenteric arteriolar vasodilatation, and determined by increased levels of vasodilators, also contributes to portal pressure increase[6].

The pathophysiology of portal hypertension in hepatosplenic mansonic schistosomiasis also displays a systemic hyperdynamic state[2]. We have previously reported that this hyperdynamic circulatory state seems to be corrected in the intraoperative period during esophagogastric devascularization and splenectomy (EGDS)[7]. However, to the best of our knowledge, there are no data in the literature regarding postoperative systemic hemodynamics after surgical treatment of schistosomal portal hypertension.

The purpose of this study was to prospectively investigate the postoperative systemic hemodynamic effects in two different surgical procedures largely employed for treatment of schistosomal portal hypertension.

MATERIALS AND METHODS

Thirty-six patients with portal hypertension and a history of previous upper digestive tract bleeding, due to esophageal varices rupture secondary to hepatosplenic mansonic schistosomiasis, were prospectively studied before and after elective surgical treatment between June 1998 and March 2005. Eighteen patients were male and eighteen female, with a mean age of 39 (range 22-56) years. Laboratory data and arterial blood gases are expressed in Table 1. Transthoracic echocardiography was performed in all patients before surgery. The Hospital Ethics Committee approved the study protocol, and all patients signed their informed consent. Immediately before surgery, patients underwent a right internal jugular vein puncture with the introduction of a pulmonary artery catheter (Edwards Swan-Ganz TM, caliber 7F, model 93A-131H; Baxter Corporation, USA) for invasive systemic hemodynamic assessment. Patients were randomized for two different elective surgical procedures: 17 were subjected to esophagogastric devascularization and splenectomy (EGDS) and 19 to distal splenorenal shunt (DSRS). A mean pulmonary artery pressure greater than 25 mmHg was considered as an absolute contraindication for DSRS.

EGDS consisted of ligation of the splenic artery close to the body of the pancreas, followed by splenectomy and devascularization of the distal 5-7 cm of the esophagus, and of the upper two thirds of the stomach proximal to the incisura angularis. DSRS consisted of dissection of the splenic vein from the splenic hilum until its junction with superior mesenteric vein, and ligating all small vessels between the pancreas and the splenic vein (splenopancreatic disconnection). Left renal vein anterior and superior surfaces were dissected, the splenic vein was transected near it's junction with the superior mesenteric vein, and an anastomosis between the splenic and renal veins was performed with a running suture. No immediate complications were observed and there was no intra- or postoperative mortality.

Four days after surgery, when the surgical effects had worn off, the systemic hemodynamic assessment was repeated and the pulmonary artery catheter was removed. There were no complications related to pulmonary artery catheterization.

Table 1 Preoperative laboratory and arterial blood gases data

	Mean ± SD	Normal values
ALT (IU/L)	31.7 ± 16.8	7-45
AST (IU/L)	31.6 ± 20.5	7-45
Gamma GT (IU/L)	46.2 ± 25.5	7-50
ALP (IU/L)	118.5 ± 46.3	60-122
BUN (mg/dL)	25.5 ± 5.75	10-50
Cr (mg/dL)	0.78 ± 0.16	0.6-1.4
TP (g/dL)	7.45 ± 0.71	6-8
ALB (g/dL)	4.18 ± 0.48	3.5-5.0
PT (s)	14.2 ± 2.9	14 ± 2
PTT (s)	29.8 ± 6.26	30 ± 2
TBIL (mg%)	1.16 ± 0.73	1.4
IBIL (mg%)	0.79 ± 0.67	0.8
Hb (g/dL)	11.2 ± 2.4	12-18
Ht (%)	34.4 ± 7.2	36-54
WBC (10^3/mm^3)	3.63 ± 2.2	4-10
PLT (10^3/mm^3)	88.74 ± 56.24	150-400
pH	7.41 ± 0.05	7.37-7.44
pO_2 (mmHg)	91.4 ± 6.5	80-100
pCO_2 (mmHg)	34.9 ± 3.2	34-45
SaO_2 (%)	97.1 ± 0.95	96-98

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Gamma GT: Gamma glutamil transpeptidase; BUN: Blood urea nitrogen; Cr: Creatinine; PT: Prothrombin time; PTT: Partial thromboplastin time; Hb: Hemoglobin; Ht: Hematocrit; WBC: White blood cells; PLT: Platelets; pO_2: Arterial oxygen tension; $PaCO_2$: Arterial carbon dioxide tension; SaO_2: Arterial oxygen saturation; ALP: Alkaline phosphatase; TP: Total serum protein; ALB: Aalbumin; TBIL: Total serum bilirubin; IBIL: Indirect bilirubin.

Statistical analysis

Statistical analysis was accomplished by the paired *t* test, and $P < 0.01$ was considered as statistically significant, with a 99% confidence interval.

RESULTS

The results of the transthoracic Doppler echocardiography are shown in Table 2. No ventricular hypertrophy or segmental contraction abnormality was observed, and no patients presented valvular lesions or pericardial effusions. All patients presented normal systolic and diastolic ventricular function. In two patients, echocardiography revealed an estimated pulmonary artery pressure of 60 and 40 mmHg, respectively, accompanied by a discrete dilatation of the right ventricle. These two patients were subjected to EGDS.

Pre- and postoperative hemodynamic evaluation data are shown in Table 3 and Figure 1. Preoperative hemodynamic evaluation revealed an increased mean cardiac index (4.78 ± 1.13 L/min per m^2) and a reduction in the systemic vascular resistance index (1457 ± 380.7 dynes.s/cm^5.m^2). The systolic index (60.24 ± 12.8 mL/beats per m^2), left cardiac work index (6.14 ± 1.43 kg.m/m^2), left ventricle systolic work index (76.6 ± 17.8 g.m/m^2), right cardiac work index (1.22 ± 0.5 kg.m/m^2) and right ventricle systolic work index (15.25 ± 6.4 g.m/m^2)

Table 2 Preoperative transthoracic echocardiography in patients with portal hypertension due to hepatosplenic mansonic schistosomiasis

	Preop (mean ± SD)	Normal range
Ao	30.7 ± 1.9	20-35 mm
LA	38.1 ± 4.6	20-40 mm
LVDD	49.7 ± 3.9	35-55 mm
FDV	125.4 ± 29.6	50-150 mL
LVSD	30.9 ± 2.8	20-35 mm
SV	30.1 ± 8.1	50-150 mL
DD	37.6 ± 3.3	30%-40%
EF	75.9 ± 4.5	65%-80%
Se	8.9 ± 0.6	7-11 mm
Pw	8.4 ± 0.5	7-11 mm
V/M	65.1 ± 6.8	45%-75%

Ao: Aorta; LA: Left atrium; LVDD: Left ventricular diastolic diameter; FDV: Final diastolic volume; LVSD: Left ventricular systolic diameter; SV: Systolic volume; DD: Shortening fraction; EF: Ejection fraction; Se: Septum wall thickness; Pw: Left ventricular wall thickness; V/M: Left ventricular volume/mass relationship; E/A: Ratio between wave E and A.

Table 3 Pre- and postoperative hemodynamic parameters in patients with portal hypertension due to hepatosplenic mansonic schistosomiasis

	Preop	EGDS	DSRS	Normal values
HR	80.2 ± 11.4	83.1 ± 10.6	86.7 ± 17.9	80-100 beats/min
MABP	91.4 ± 12.5	93.6 ± 14.4	92.36 ± 13.75	80-100 mmHg
RAP	7.9 ± 2.5	7 ± 2.4	7.26 ± 2.4	0-7 mmHg
PCWP	10.2 ± 2.7	9.1 ± 3	9.55 ± 2	8-12 mmHg
PAP	18 ± 5.1	12.65 ± 4.7[d]	19.84 ± 5.2	12-15 mmHg
CI	4.78 ± 1.13	3.8 ± 0.4[d]	5.2 ± 0.76	2.5-4 L/min per m^2
SI	60.24 ± 12.8	46.2 ± 8.6[d]	59.64 ± 10.5	41-51 mL/beat per m^2
SVRI	1457 ± 380.7	1901.4 ± 330.2[d]	1389 ± 311	1970-2390 dynes.s/cm^5.m^2
PVRI	133 ± 62	141.65 ± 102.9	139.7 ± 67.8	225-315 dynes.s/cm^5.m^2
LCWI	6.14 ± 1.43	4.9 ± 0.7[b]	6.94 ± 1.3	3.4-4.2 kg.m/m^2
LVSWI	76.6 ± 17.8	59 ± 12.6[b]	82.65 ± 17	50-62 g.m/m^2
RCWI	1.22 ± 0.5	0.79 ± 0.4[b]	1.31 ± 0.35	0.54-0.60 kg.m/m^2
RVSWI	15.25 ± 6.4	9.45 ± 4.8[b]	16.3 ± 6.3	7.9-9.7 g.m/m^2

[b]P < 0.01 between EGDS and PREOP; [d]P < 0.001 between EGDS and PREOP. Values are expressed as means ± SD. EGDS: Esophagogastric devascularization and splenectomy; DSRS: Distal splenorenal shunt; HR: Heart rate; MABP: Mean arterial blood pressure; PAP: Mean pulmonary artery pressure; RAP: Mean right atrium pressure; PCWP: Pulmonary capillary wedged pressure; CI: Cardiac index; SI: Systolic index; SVRI: Systemic vascular resistance index; PVRI: Pulmonary vascular resistance index; RCWI and LCWI: Right and left cardiac work indexes, LVSWI and RVSWI: Left and right ventricular systolic work indexes.

were all increased. Heart rate (80.2 ± 11.4 beats/min), mean arterial blood pressure (91.4 ± 12.5 mmHg) and pulmonary capillary wedge pressure (10.2 ± 2.7 mmHg) were all within normal limits. Mean pulmonary artery pressure (18 ± 5.1 mmHg), as well as right atrial pressure (7.9 ± 2.5 mmHg), was increased, while the pulmonary vascular resistance index (133 ± 62 dynes.s/cm^5.m^2) was decreased.

Four days after EGDS, there was a significant decrease in cardiac index (3.8 ± 0.4 L/min per m^2) and a significant increase in systemic vascular resistance index (1901.4 ± 330.2 dynes.s/cm^5.m^2) toward normal levels. The systolic index (46.2 ± 8.6 mL/beats per m^2), left cardiac work index

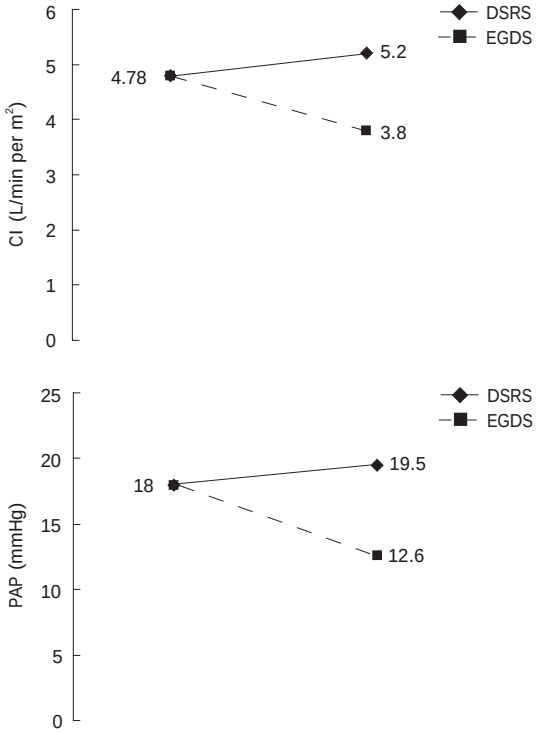

Figure 1 Schematic illustration showing important differences in cardiac index (CI) and pulmonary artery pressure (PAP) before and after EGDS (dashed line) and DSRS (continuous line).

(4.9 ± 0.7 kg.m/m^2) and left ventricle systolic work index (59 ± 12.6 g.m/m^2) also significantly decreased toward normal levels. There was no significant alteration in heart rate (83.1 ± 10.6 beats/min), mean arterial blood pressure (93.6 ± 14.4 mmHg), pulmonary capillary wedge pressure (9.1 ± 3 mmHg) and right atrial pressure (7 ± 2.4 mmHg). There was also a significant reduction in pulmonary artery pressure (12.65 ± 4.7 mmHg), right cardiac work index (0.79 ± 0.4 kg.m/m^2), right ventricle systolic work index (9.45 ± 4.8 g.m/m^2), and a non-significant increase in pulmonary vascular resistance index (141.65 ± 102.9 dynes.s/cm^5.m^2).

Four days after DSRS, there was a non-significant increase in cardiac index (5.2 ± 0.76 L/min per m^2), and a non-significant decrease in systemic vascular resistance index (1389 ± 311 dynes.s/cm^5.m^2). The systolic index (59.64 ± 10.5 mL/beats per m^2), left cardiac work index (6.94 ± 1.3 kg.m/m^2) and left ventricle systolic work index (82.65 ± 17 g.m/m^2) remained above normal levels. There were non-significant increases in heart rate (86.7 ± 17.9 beats/min) and mean arterial blood pressure (92.36 ± 13.75 mmHg), and non-significant decreases in pulmonary capillary wedge pressure (9.55 ± 2 mmHg) and right atrial pressure (7.26 ± 2.4 mmHg). In addition, there were non-significant increases in pulmonary artery pressure (19.84 ± 5.2 mmHg), right cardiac work index (1.31 ± 0.35 kg.m/m^2), and right ventricle systolic work index (16.3 ± 6.3 g.m/m^2), with no significant alteration in pulmonary vascular resistance index (139.7 ± 67.8 dynes.s/cm^5.m^2).

DISCUSSION

Hemodynamic changes after the surgical treatment of schistosomal portal hypertension with disconnection or

shunt procedures may contribute to the understanding of the hyperdynamic circulation observed in these patients with portal hypertension and preserved liver function. Twenty-nine patients (80.5%) showed an elevated cardiac index in the preoperative evaluation, which characterized a hyperdynamic circulatory state[8,9]. Although easily clinically recognized in patients with cirrhosis by peripheral arterial vasodilatation, hypotension and tachycardia, which are usually related to progressive liver failure, in schistosomal patients, these alterations are completely absent because they are less intense and liver function is preserved[10].

We have previously demonstrated that hepatosplenic schistosomiasis presents mild pulmonary hypertension and induces a hyperdynamic circulatory state, which is corrected after EGDS[2,7]. We have suggested that these changes are correlated with the portosystemic collateral circulation, especially as a consequence of splanchnic hyperflow[7]. Our group, as do Wattanasirichaigoon *et al*[8], believes that portosystemic collateral circulation mimics an arteriovenous fistula, in which the high-pressure portal blood connects with the lower pressure systemic venous circulation, which decompresses the portal circulation, but increases portal blood flow. As portal blood flow increases, so does collateral flow, and it is almost totally shunted with the systemic circulation. These observations should be considered particularly in patients with hepatosplenic schistosomiasis, in whom large splenomegaly induces splenic hyperflow, which plays an important role in the origin and maintenance of hyperdynamic circulation[3]. In the present study, 15 patients subjected to EGDS (88.2%) presented normalization of cardiac and systemic vascular resistance indexes, which suggested that EGDS corrected the hyperdynamic circulation. In cirrhosis, physical exercise and pharmacological stress determine an increase in left ventricular end diastolic pressure and a fall in cardiac stroke index and left ventricular ejection fraction, which indicates an abnormal ventricular response[9,11,12]. In these patients, the reduced vascular resistance may mask left ventricular failure. In contrast, the correction of hyperdynamic circulation without elevation of filling pressure, and the normal pre-operative echocardiographic parameters strongly suggest an absence of cardiomyopathy in patients with portal hypertension due to hepatosplenic mansonic schistosomiasis.

On the other hand, patients subjected to DSRS maintained a hyperdynamic circulation. These observations suggested that splenic flow through the venous shunt maintained a low-resistance circuit and hence, the hyperdynamic circulation. These findings are corroborated by the increases in heart rate, systolic, left cardiac work and left ventricle systolic work indexes observed in these patients. Studies that assess the hemodynamic pattern in a later period after EGDS or DSRS are necessary to confirm the findings of our study.

Portopulmonary hypertension (PPHTN) is defined as an increase in mean pulmonary artery pressure greater than 25 mmHg, increased pulmonary vascular resistance (greater than 120 dynas/cm^5), and pulmonary capillary wedge pressure lower than 15 mmHg, in the presence of portal hypertension[13-15]. There are several mechanisms proposed for its development, including an increased production of vasoconstrictors[16-18], increased pulmonary blood flow leading to vascular endothelial damage and remodeling[19], excess of pulmonary vascular volume[20], cirrhotic cardiomyopathy with myocardial thickening and diastolic dysfunction[21], and in situ microthrombosis[22]. Interestingly, to date there are no data showing any correlation between the extent of PPHTN and the intensity of portal pressure, severity of liver disease or degree of shunting[23].

Arterial blood gases and left (systolic and diastolic) ventricular function were within normal limits and, pulmonary capillary wedge pressure was < 15 mmHg in all patients during the hemodynamic study, which provided evidence of normal cardiopulmonary function.

In the present study, 23 patients (63.8%) presented with pulmonary artery pressure greater than 15 mmHg; 13 (36.1%) between 15 and 20 mmHg, eight (22.2%) between 20 and 25 mmHg. In two patients (5.5%), pulmonary artery pressure was > 25 mmHg, which demonstrated a tendency toward pulmonary hypertension in schistosomal portal hypertension, as previously described by our group[24].

After EGDS, we observed a significant reduction in pulmonary artery pressure in 88.2% of the patients. There was also a significant reduction in right cardiac work and right ventricular systolic work indexes, without significant increase in pulmonary vascular resistance index. The two patients without pulmonary artery pressure reduction showed a normal preoperative pulmonary artery pressure (< 15 mmHg). These findings suggest that pulmonary hyperflow may contribute to the elevated pulmonary artery pressure observed in these patients. The reduced pulmonary vascular resistance may be an accommodation of pulmonary vasculature to the hyperflow, in an attempt to maintain normal pulmonary artery pressure. Nevertheless, this adaptation seems ineffective, since pulmonary artery pressure was elevated in the majority of the patients studied. The two patients with PPHTN subjected to EGDS showed reduction of both cardiac index and mean pulmonary artery pressure.

These findings were confirmed by the hemodynamic pattern of patients subjected to DSRS, with which, 16 patients (84.2%) showed a slight increase in pulmonary artery pressure, right cardiac work and right ventricular systolic work indexes. In fact, we have previously reported the cases of two young asymptomatic patients with normal cardiovascular preoperative assessment (electrocardiography, thoracic X-ray and transthoracic echocardiography) who died 4 and 7 d after DSRS, and necropsy showed signs of acute pulmonary hypertension and right ventricular failure[25]. We hypothesized that these patients had undiagnosed preoperative elevated mean pulmonary artery pressure that caused acute pulmonary hypertension after the splenorenal shunt, due to pulmonary hyperflow. The importance of preoperative hemodynamic evaluation in hepatosplenic schistosomiasis is to identify patients with raised pulmonary artery pressure, and consequently, to choose EGDS rather than DSRS as the ideal surgical treatment.

In conclusion, the hyperdynamic circulatory state present in hepatosplenic mansonic schistosomiasis was corrected by EGDS, but it was maintained by DSRS.

Similarly, the raised mean pulmonary artery pressure was corrected by EGDS and maintained by DSRS. EGDS seems to be the most physiologic surgical alternative for patients with schistosomal portal hypertension, because of its tendency to lead to immediate normalization of the systemic and pulmonary hemodynamic parameters. Cardiomyopathy present in cirrhotic portal hypertension seems to be absent in hepatosplenic mansonic schistosomiasis. Hemodynamic studies to evaluate the late circulatory pattern in these patients are necessary to confirm our findings.

COMMENTS

Background

To the best of our knowledge, there are no data in the literature regarding postoperative systemic hemodynamics after surgical treatment of schistosomal portal hypertension. The purpose of this study was to prospectively investigate the postoperative systemic hemodynamic effects in two different surgical procedures largely employed for treatment of schistosomal portal hypertension.

Research frontiers/Innovations and breakthroughs

Hemodynamic changes after the surgical treatment of schistosomal portal hypertension with disconnection or shunt procedures may contribute to the understanding of the hyperdynamic circulation observed in these patients with portal hypertension and preserved liver function. This knowledge may be useful in deciding on the best surgical option for each patient.

Applications

The importance of preoperative hemodynamic evaluation in hepatosplenic schistosomiasis is to identify patients with raised pulmonary artery pressure, and consequently, choose EGDS rather than DSRS as the ideal surgical treatment. EGDS seems to be the most physiologic surgical alternative for patients with schistosomal portal hypertension, due to its tendency to lead to immediate normalization of the systemic and pulmonary hemodynamic parameters.

Terminology

Schistosomal portal hypertension: presinusoidal portal hypertension in patients with preserved liver function.

Peer review

This study examines in patients with pre-hepatic portal hypertension, caused by schistosomiasis, the short-term effects of two different interventions, EGDS and DSRS, on hemodynamic cardiac parameters derived from transthoracic echocardiography and direct measurement of pulmonary artery pressure. The study shows that EGDS lowers cardiac output and mean pulmonary artery pressure (probably as a result of splenectomy), while DSRS has no effect on these parameters.

REFERENCES

1 **Silva LC**. Schistosomiasis mansoni. Clinical features. In: Okuda K, Benhamou J-P, editors. Portal hypertension: clinical and physiological aspects. Tokyo: Springer-Verlag, 1991: 309-318
2 **de Cleva R**, Pugliese V, Zilberstein B, Saad WA, Pinotti HW, Laudanna AA. Hyperdynamic circulation in Manson's hepatosplenic schistosomiasis. *Rev Hosp Clin Fac Med Sao Paulo* 1998; **53**: 6-10
3 **Cleva R**, Saad WA, Herman P, Pugliese V, Zilberstein B, Laudanna AA, Gama-Rodrigues JJ. Portal hyperflow in patients with hepatosplenic mansonic schistosomiasis. *Rev Hosp Clin Fac Med Sao Paulo* 2004; **59**: 10-14
4 **de Cleva R**, Herman P, Saad WA, Pugliese V, Zilberstein B, Rodrigues JJ, Laudanna AA. Postoperative portal vein thrombosis in patients with hepatosplenic mansonic schistosomiasis: relationship with intraoperative portal pressure and flow. A prospective study. *Hepatogastroenterology* 2005; **52**: 1529-1533
5 **Garcia-Tsao G**. Portal hypertension. *Curr Opin Gastroenterol* 2004; **20**: 254-263
6 **Tsai MH**, Iwakiri Y, Cadelina G, Sessa WC, Groszmann RJ. Mesenteric vasoconstriction triggers nitric oxide overproduction in the superior mesenteric artery of portal hypertensive rats. *Gastroenterology* 2003; **125**: 1452-1461
7 **de Cleva R**, Pugliese V, Zilberstein B, Saad WA, Pinotti HW, Laudanna AA. Systemic hemodynamic changes in mansonic schistosomiasis with portal hypertension treated by azygoportal disconnection and splenectomy. *Am J Gastroenterol* 1999; **94**: 1632-1637
8 **Wattanasirichaigoon S**, Gordon FD, Resnick RH. Hyperdynamic circulation in portal hypertension: a comparative model of arterio-venous fistula. *Med Hypotheses* 2000; **55**: 77-87
9 **Moller S**, Henriksen JH. Cirrhotic cardiomyopathy: a pathophysiological review of circulatory dysfunction in liver disease. *Heart* 2002; **87**: 9-15
10 **de Cleva R**, Genzini T, Laudanna AA. Hipertensão portal na Esquistossomose. *Revista de Medicina de São Paulo* 1996; **75**: 126-129
11 **Ruiz-del-Arbol L**, Monescillo A, Jimenez W, Garcia-Plaza A, Arroyo V, Rodes J. Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997; **113**: 579-586
12 **Kelbaek H**, Rabol A, Brynjolf I, Eriksen J, Bonnevie O, Godtfredsen J, Munck O, Lund JO. Haemodynamic response to exercise in patients with alcoholic liver cirrhosis. *Clin Physiol* 1987; **7**: 35-41
13 **Benjaminov FS**, Prentice M, Sniderman KW, Siu S, Liu P, Wong F. Portopulmonary hypertension in decompensated cirrhosis with refractory ascites. *Gut* 2003; **52**: 1355-1362
14 **Castro M**, Krowka MJ, Schroeder DR, Beck KC, Plevak DJ, Rettke SR, Cortese DA, Wiesner RH. Frequency and clinical implications of increased pulmonary artery pressures in liver transplant patients. *Mayo Clin Proc* 1996; **71**: 543-551
15 **Mandell MS**. Critical care issues: portopulmonary hypertension. *Liver Transpl* 2000; **6**: S36-S43
16 **Kiely DG**, Cargill RI, Struthers AD, Lipworth BJ. Cardiopulmonary effects of endothelin-1 in man. *Cardiovasc Res* 1997; **33**: 378-386
17 **Panos RJ**, Baker SK. Mediators, cytokines, and growth factors in liver-lung interactions. *Clin Chest Med* 1996; **17**: 151-169
18 **Higenbottam T**. Pathophysiology of pulmonary hypertension. A role for endothelial dysfunction. *Chest* 1994; **105**: 7S-12S
19 **Liu H**, Lee SS. Cardiopulmonary dysfunction in cirrhosis. *J Gastroenterol Hepatol* 1999; **14**: 600-608
20 **Krowka MJ**. Hepatopulmonary syndrome versus portopulmonary hypertension: distinctions and dilemmas. *Hepatology* 1997; **25**: 1282-1284
21 **De BK**, Majumdar D, Das D, Biswas PK, Mandal SK, Ray S, Bandopadhyay K, Das TK, Dasgupta S, Guru S. Cardiac dysfunction in portal hypertension among patients with cirrhosis and non-cirrhotic portal fibrosis. *J Hepatol* 2003; **39**: 315-319
22 **Herve P**, Lebrec D, Brenot F, Simonneau G, Humbert M, Sitbon O, Duroux P. Pulmonary vascular disorders in portal hypertension. *Eur Respir J* 1998; **11**: 1153-1166
23 **Hadengue A**, Benhayoun MK, Lebrec D, Benhamou JP. Pulmonary hypertension complicating portal hypertension: prevalence and relation to splanchnic hemodynamics. *Gastroenterology* 1991; **100**: 520-528
24 **de Cleva R**, Herman P, Pugliese V, Zilberstein B, Saad WA, Rodrigues JJ, Laudanna AA. Prevalence of pulmonary hypertension in patients with hepatosplenic Mansonic schistosomiasis--prospective study. *Hepatogastroenterology* 2003; **50**: 2028-2030
25 **de Cleva R**, Herman P, Pugliese V, Zilberstein B, Saad WA, Gama-Rodrigues JJ. Fathal pulmonary hypertension after distal splenorenal shunt in schistosomal portal hypertension. *World J Gastroenterol* 2004; **10**: 1836-1837

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RAPID COMMUNICATION

Neural cell adhesion molecule-180 expression as a prognostic criterion in colorectal carcinoma: Feasible or not?

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Abstract

AIM: To evaluate the frequency of neural cell adhesion molecule (NCAM)-180 expression in fresh tumor tissue samples and to discuss the prognostic value of NCAM-180 in routine clinical practice.

METHODS: Twenty-six patients (16 men, 10 women) with colorectal cancer were included in the study. Fresh tumor tissue samples and macroscopically healthy proximal margins of each specimen were subjected to flow-cytometric analysis for NCAM-180 expression.

RESULTS: Flow-cytometric analysis determined NCAM-180 expression in whole tissue samples of macroscopically healthy colorectal tissues. However, NCAM-180 expression was positive in only one case (3.84%) with well-differentiated Stage Ⅱ disease who experienced no active disease at 30 mon follow-up.

CONCLUSION: As a consequence of the limited number of cases in our series, it might not be possible to make a generalisation, nevertheless the routine use of NCAM-180 expression as a prognostic marker for colorectal carcinoma seems to be unfeasible and not cost-effective in clinical practice due to its very low incidence.

Key words: Neural cell adhesion molecule-180; Colorectal cancer; Prognosis; Flow-cytometry

INTRODUCTION

Cancer is currently one of the major causes of morbidity and mortality in humans. Tumor progression to local invasion and metastasis are clinically the most relevant processes for prognosis. However the molecular pathways involved in tumor progression are the least well defined at the cellular level, which represents one of prime challenges in cancer research. Tumor suppressor genes are the major target for treatment modalities in most malignant diseases, including gastrointestinal neoplasies. For colon carcinoma, Deleted in Colon Carcinoma (DCC) accounts for one of the best described tumor suppressors involved in adhesive interactions. DCC is a member of the immunglobulin (Ig) superfamily. The neural cell adhesion molecule (NCAM, CD56) is another member of this family possessing structural and sequence homology to DCC[1,2]. Members of the Ig family of cell adhesion molecules (CAMs) play an important role in progression to tumour malignancy and metastasis. NCAM is an embryologic adhesion molecule and a cell membrane protein that modulates neuroendocrine cell growth, migration, and differentiation[3]. NCAM mediates cell-cell and cell-matrix adhesion, contact inhibition and tissue morphogenesis and also is proposed to be critical in signal transduction[3,4]. The major variants of NCAM are classified based on the sialic acid content as either NCAM-H (high-sialic-acid content) or NCAM-L (low-sialic-acid content). The properties of NCAM-H molecules are the following: relative molecular weight between 200-250 kDa, more prevelant in embrionic tissue, blocks adhesion-binding sites and fascilitates cell migration during embriogenesis[5-7]. Therefore, cell-cell or cell-matrix adhesions can be altered by downregulation of NCAM molecules or by upregulation of sialic acid content within the NCAM protein. NCAM-L with a molecular weight of 120-180 kDa predominates in adult tissue and is expressed in three major isoforms, resulting from alternative mRNA splicing and depending on cell type and stage of differentiation[5,8,9]. The major

isoforms have the 5-distal immunoglobulin and 2-membrane proximal fibronectin (FN)-Ⅲ domains. NCAM-120 is glycophosphatidylinositol-linked to the plasma membrane by a sequence encoded by exon 15[9]. NCAM-140 has the basic NCAM-120 structure with a transmembrane sequence and a short (40-kDa) intracellular tail. NCAM-180 has a longer intracellular tail (90 kDa) encoded by exons 17, 19, and unique to this isoform, exon 18. The intracellular component of NCAM-180 anchors the molecule to the cytoskeleton. NCAM-180 is believed to be an important structural molecule that mediates cell-cell adhesion by providing a mechanical linkage between the cytoskeleton and the extracellular adhesive end of the molecule resulting in tissue stabilisation[10]. NCAM-180 was found to be expressed in normal colonic epithelium villous tips and the expression was demonstrated to be lost in highly aggressive colon cancers[7,11]. This study was undertaken to further evaluate the frequency of NCAM-180 expression in fresh tumor tissue samples by flow-cytometric analysis and to discuss the prognostic value of NCAM-180 in colorectal carcinoma in routine clinical practice.

MATERIALS AND METHODS

Patients and tumor samples

Fresh tumor tissue samples were obtained at operation from 26 patients with colorectal cancer who underwent surgery between January 2002 and January 2006. Two samples from each case, one of which was chosen directly from the center of a main tumor lesion and the other from the macroscopically healthy proximal margins, were transferred to flow-cytometric analysis immediately. The remaining specimen was fixed in 10% phosphate buffered formaldehyde, and embedded in paraffin for histopathological analysis. Patient characteristics are shown in Table 1. Oncologic follow-up was performed in each case within 6-12 mo periods. Clinical data were obtained by direct interviews with patients as a part of oncologic follow-up. Patients were defined as having an aggressive clinical course if they presented with an obstructing or perforating lesion or had metastatic disease. Death within 18 mo of presentation was also classified as having an aggressive clinical course. Participation in the study was voluntary and all patients gave their informed consent to participate. The study was approved by the Local Ethics Committee of Zonguldak Karaelmas University Hospital, Zonguldak, Turkey.

Table 1 Background of 26 cases of resected colorectal carcinoma

Case	Gender	Age (yr)	Location	Tumour
1	F	49	Colon	Adenocarcinoma
2	F	70	Rectum	Adenocarcinoma
3	M	86	Colon	Adenocarcinoma
4	M	76	Colon	Adenocarcinoma
5	M	73	Colon	Adenocarcinoma
6	F	76	Colon	Adenocarcinoma
7	M	72	Colon	Adenocarcinoma
8	F	68	Colon	Adenocarcinoma
9	M	72	Colon	Adenocarcinoma
10	M	50	Rectum	Adenocarcinoma
11	M	88	Colon	Adenocarcinoma
12	F	68	Rectum	Adenocarcinoma
13	F	48	Colon	Adenocarcinoma
14	F	75	Rectum	Adenocarcinoma
15	M	37	Rectum	Adenocarcinoma
16	M	57	Rectum	Adenocarcinoma
17	F	71	Colon	Adenocarcinoma
18	M	70	Colon	Adenocarcinoma
19	M	47	Colon	Adenocarcinoma
20	M	47	Colon	Adenocarcinoma
21	F	71	Colon	Adenocarcinoma
22	M	53	Colon	Adenocarcinoma
23	M	80	Rectum	Adenocarcinoma
24	F	49	Rectum	Adenocarcinoma
25	M	76	Colon	Adenocarcinoma
26	M	62	Rectum	Adenocarcinoma

Flow-cytometric analysis

All biopsy materials were dissociated mechanically with Medimachine (Becton Dickinson, CA, USA). The dissociated cells were prepared as single cell suspension in PBS (phosphate buffered salt solution) .The cell number was calibrated as 10×10^6/mL. Each 100 μL sample incubated with 10 μL anti-CD56-PE (phycoerythrin conjugated NCAM monoclonal antibody) for 15 min at room temperature. Samples were processed by a Coulter Q Prep Workstation and run with a Beckman-Coulter Epics XL MCL flow cytometer (Beckman coulter, Florida, USA). At least 20000 events were acquired for each sample. Data analysis was performed using EXPO32 (Beckman-Coulter) software. Only CD45 negative population gated were used for NCAM analysis. The upper limit of background fluorescence was set such that no more than 1% of the events with the matched isotype was in the positive region.

Histological classification

Pathologic stagings were performed based on the TNM staging system developed by the American Joint Committee on Cancer[12]. Histologic tumor typing was applied according to the classification system indicating poor, moderate or well differentiation. Macroscopically healthy proximal margins were verified to be tumor free by histopathologic examination.

RESULTS

Of the 26 patients, 16 (61.5%) were male and 10 (38.5%) were female. The mean age was 65.04 ± 13.60 (range, 37-88) years. Tumors were found to be localized in colonic segments in 19 (73.07%) and in rectum in the rest 7 (26.93%) cases. Four patients died because of cardiovascular or pulmonary complications following surgery. No patients died during follow-up. The mean follow-up period was 19.05 ± 12.33 (range, 4-56) mo. Histopathologic stage, differentiation status, NCAM-180 expression and postoperative survival periods are shown in Table 2. The number of patients in Stage Ⅰ, Ⅱ, Ⅲ and Ⅳ disease were 3 (11.53%), 7 (26.92%), 9 (34.61%), and 7 (26.92%), respectively. Tumors were detected to be well-differentiated in 4 (15.38%), moderately-differentiated in 15 (57.69%) and poorly-differentiated in 7 (26.92%) cases. Flow-cytometric analysis determined NCAM-180 expression in whole tissue samples of macroscopically

Table 2 Results of histopathologic evaluation and flow cytometric analysis of NCAM-180 status

Case	pTNM	Stage	Histology	NCAM-180	Outcome
1	T3N0M0	Ⅱ	Moderate	-	No active disease at 21 mo follow-up
2	T4N1M1	Ⅳ	Poor	-	Died of metastatic diesease 8 mo postresection
3	T4N2M1	Ⅳ	Moderate	-	No active disease at 9 mo follow-up
4	T3N0M0	Ⅱ	Well	-	No active disease at 20 mo follow-up
5	T4NIM1	Ⅳ	Poor	-	Died of cardiopulmonary complication postoperatively
6	T3N1M0	Ⅲ	Moderate	-	No active disease at 6 mo follow-up
7	T2N0M0	Ⅰ	Moderate	-	Metachrone colonic disease at 15 mo
8	T2N0M0	Ⅰ	Moderate	-	No active disease at 15 mo follow-up
9	T4N2M0	Ⅲ	Moderate	-	Died of metastatic diesease 18 mo postresection
10	T3N2M0	Ⅲ	Moderate	-	No active disease at 18 mo follow-up
11	T2N0M0	Ⅰ	Moderate	-	Died of cardiopulmonary complication postoperatively
12	T3N0M1	Ⅳ	Poor	-	Died of cardiopulmonary complication postoperatively
13	T3N1M0	Ⅲ	Moderate	-	No active disease at 32 mo follow-up
14	T3N1M0	Ⅲ	Moderate	-	No active disease at 20 mo follow-up
15	T3N0M0	Ⅱ	Poor	-	No active disease at 16 mo follow-up
16	T3N1M0	Ⅲ	Well	-	No active disease at 19 mo follow-up
17	T3N0M0	Ⅱ	Well	+	No active disease at 30 mofollow-up
18	T3N1M0	Ⅲ	Well	-	No active disease at 44 mo follow-up
19	T4N2M1	Ⅳ	Poor	-	Died of metastatic diesease 5 mo postresection
20	T4N2M1	Ⅳ	Poor	-	Died of metastatic diesease 4 mo postresection
21	T3N0M0	Ⅱ	Moderate	-	Died of metastatic diesease 56 mo postresection
22	T4N1M1	Ⅳ	Moderate	-	Died of metastatic diesease 15 mo postresection
23	T4N1M0	Ⅲ	Moderate	-	Died of cardiopulmonary complication postoperatively
24	T3N0M0	Ⅱ	Poor	-	No active disease at 18 mo follow-up
25	T3N0M0	Ⅱ	Moderate	-	No active disease at 16 mo follow-up
26	T4N1M0	Ⅲ	Moderate	-	No active disease at 14 mo follow-up

healthy colorectal tissues. However, NCAM-180 expression was positive in only one case (3.84%) with well-differentiated Stage Ⅱ disease, and this patient experienced no active disease at 30 mo follow-up.

Correlation between NCAM-180 expression in colorectal cancer and other parameters

It is not possible to compare overall survival outcomes in this series with only one (3.84%) positive NCAM-180 expression. However, NCAM-180 expression was positive in a well-differentiated Stage Ⅱ tumor with an uneventfull clinical course for 30 mo following surgery. Considering well-differentiated tumors, one of three patients without NCAM-180 expression experienced a longer disease free survival period (44 *vs* 30 mo). Moreover, NCAM-180 epxression was not detected in both moderate or poor differentiated tumors. Evaluation of the patients with stage Ⅱ disease demonstrated that one of six patients without NCAM-180 expression survived 56 mo after diagnosis and no active disease was detected in the other 5 patients within a mean follow-up period of 18.2 (range, 16-21) mo.

DISCUSSION

Tumoral invasion and metastasis are the most critical and complex processes in aggressive human cancers and are one of the major causes of cancer deaths. Cell adhesion molecules, including the immunoglobulin superfamily, play a crucial role in determining tumor development and the metastatic cascade[13,14]. Variations in cell-cell and cell-matrix adhesion accompany the progression from benign tumours to invasive, malignant cancer and the subsequent metastatic dissemination of tumour cells. The hallmark of neoplastic and metastatic growth is thought to be reduced adhesiveness between cells and also between cells and the extracellular matri[3]. Several groups of adhesion molecules are importantly involved in regulation of tumor invasion and metastasis.

NCAM (CD56) is a calcium independent cell adhesion molecule, which mediates homotypic and heterotypic cell-cell and cell-matrix adhesion[15-17]. NCAM has been found to be a significant factor for survival in various solid tumors. A correlation between reduced NCAM expression and poor prognosis has been reported for some cancer types[11,18,19]. The existence of NCAM-180 has been proposed to be a good prognostic criterion in colorectal carcinoma[11]. Previous studies have demonstrated that NCAM-180 is present in normal colonic epithelium and in benign colonic tumors and loss of NCAM-180 expression might result in defective intracellular adhesion between colonocytes in aggressive colon carcinoma[7,11]. In this study we investigated the NCAM-180 expression rate in fresh tumor tissue samples of colorectal carcinoma and the association of an aggresive clinical course with loss of this expression.

NCAM expression has been investigated in various solid and neuroendocrine tumours. There is a consensus that presence of its polisialiated (embryonic) form, which is less adhesive than the adult form [that contains a relatively low polisialic acid (PSA) content], is associated with a poor prognosis. Correlation between N-CAM expression and perineural spread has been confirmed in a variety of human carcinomas. The existence of the polisialiated form of NCAM in Wilms' tumor, neuroblastoma, pituitary tumor, small cell lung cancer, gallbladder and bile duct cancer,

squamous cell cancer of head and neck, and prostat cancer results in perineural invasion and agrressive metastatic behaviour with a poor clinical outcome[20-28]. As the expression of the polisialiated form of NCAM correlates with tumor growth and invasiveness because of its role in cell disassociation, ıt was considered to be a poor prognostic criterion in pituitary tumors and rhabdomyosarcoma[22,29]. Polysialation has been proposed to involve steric inhibition of membrane-membrane apposition and cell adhesiveness, based on the biophysical properties of the polisialic acid[30]. In renal cell carcinoma, NCAM expression was suggested to be a risk factor for tumor metastasis[31]. Moreover, NCAM is not polysialylated in renal cell carcinoma suggesting that it plays another role in these tumors involving homophilic adhesion[31]. Conversely, for other tumors like pancreatic adenocarcinomas, reduced levels of NCAM expression were found to correlate with increased tumor malignancy[19]. This result was also observed in a transgenic mouse model of β-cell pancreatic carcinoma by crossing these mice with NCAM knockout mice[32]. The hypothesis was reduced levels of NCAM could increase cell dissociation from primary tumors. Moreover, an overall decrease in the NCAM level has been observed in another subset of tumors including colon carcinoma and astrocytoma. In these tumors NCAM expression is markedly down-regulated, and the loss of NCAM correlates with poor prognosis[7,11,18,33]. In gastrointestinal neoplasia, when pancreatic, colorectal and gastric cancer were considered, poorly differentiated tumors had lower levels of NCAM than well or moderately differentiated tumors[18].

Previous studies have demonstrated that NCAM-180 is present in normal colonic epithelium and NCAM-180 expression was found to be absent in clinically aggressive colon carcinomas[11]. Consistent with this thesis, colorectal carcinomas expressing NCAM-180 should experience a good clinical course with longer disease free survival. In other words, overexpression of the polysialylated form of NCAM or reduced expression of NCAM-180 has been suggested to decline intracellular adhesion, facilitating metastatic behavior in cancer. This study was designed to determine the rate of NCAM-180 expression in fresh colorectal tumour tissue and correlation of NCAM-180 expression with clinical course. In our series of 26 colorectal carcinoma, we determined NCAM-180 expression in only one patient (3.84%) (pathologic stage Ⅱ-well differentiated tumour) with a good clinical course during a follow-up period of 30 mo. This was an expected finding according to the previous literature[7,11]. However, we detected that 6 of the other patients with the same clinical and pathological stage at diagnosis and surgery, experienced either similar or a better clinical course during follow-up as well. Moreover, 2 patients without NCAM-180 expression and in an advanced pathological stage at diagnosis survived more than the patient with NCAM-180 expression. These are controversial results predicting that attribution of NCAM-180 expression as a good prognostic criterion in colorectal carcinoma is something to be interrogated before acceptance.

NCAM-180 has been proposed as a candidate tumor supressor in colorectal carcinoma previously and might play a crucial role in tumor behaviour by mediating colonic epithelial integrity and preventing tumour invasiveness and metastasis due to cellular adhesive properties. When colorectal cancer is considered, loss of NCAM-180 expression might lead to reduced homotypic binding between cancerous cells, resulting in detachment from the primary cancerous mass and invading other organs, acting systematically. However, in our series the NCAM-180 expression rate was only 3.84% and statistical correlation analysis of survival with NCAM-180 expression was not possible according to this low frequency. Moreover, the comparision according to tumor differentiation and stage revealed that loss of NCAM-180 expression in either well-differentiated or stage Ⅱ disease did not result in a worst clinical course. As a consequence of the limited number of cases in our series, it might not be possible to make a generalisation, nevertheless the routine use of NCAM-180 expression as a prognostic marker for colorectal carcinoma seems not to be feasible and cost-effective in clinical practice due to being present at a very low frequency. Further studies with a greater number of cases are thus called for to study the underlying mechanisms of tumor metastasis and prognosis in colorectal carcinoma.

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COMMENTS

Background

Cancer being one of the most mortal disease worldwide, tumor markers and prognostic criterions attract a great enthusiasm above researchers. Tumor suppressor genes and cell adhesion molecules are considered to play a crucial role in tumor pathophysiology.

Research frontiers

Neural cell adhesion molecule (NCAM-CD56) mediates cell-cell and cell-matrix adhesion, contact inhibition and tissue morphogenesis and also proposed to be critical in signal transduction. The major variants of NCAM are classified based on the sialic acid content as either NCAM-H (high-sialic-acid content) or NCAM-L (low-sialic-acid content). NCAM-L with a molecular weight of 120-180 kDa, predominates in adult tissue and is expressed in three major isoforms. NCAM-180 is believed to be an important structural molecule that mediates cell-cell adhesion by providing a mechanical linkage between the cytoskeleton and the extracellular adhesive end of the molecule resulting in tissue stabilisation.

Innovations and breakthroughs

A correlation between reduced NCAM expression and poor prognosis has been reported for some cancer types. NCAM-180 expression has been demonstrated to be lost in highly aggressive colon cancer and proposed to function as a tumor supressor. From this point of view we aim to evaluate the frequency of NCAM-180 expression in fresh tumor tissue samples by flow-cytometric analysis and to discuss the prognostic value of NCAM-180 in colorectal carcinoma in routine clinical practice.

Applications

The most critical deficit in the ability to treat cancer effectively is the lack of knowledge about cellular basis and markers for early diagnosis. The verification of an association between various types of malignancies and adhesion molecules might provide novel targets to cancer therapy by indicating the accurate goals.

Terminology

Neural cell adhesion molecule (NCAM-CD56) is a well identified cell membrane protein and a member of immunoglobulin superfamily, possessing structural and sequence resemblance to Deleted in Colon Carcinoma (DCC), which is another member of the same superfamily.

Peer review

The authors evaluated the frequency of NCAM-180 expression in fresh tumor tissue samples by flow-cytometric analysis and found that NCAM-180 expression in whole tissue samples of macroscopically healthy colorectal tissues, but only in one case (3.84%) with well-differentiated Stage Ⅱ disease. As discussed by the authors that the limited number of cases in the series, it is impossible to make a generalization. Further study with a large series of cases should be carried out to evaluated the clinicopathological significance of NCAM-180 expression in colorectal cancers.

REFERENCES

1 **Fearon ER**, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 1990; **247**: 49-56

2 **Fearon ER**, Pierceall WE. The deleted in colorectal cancer (DCC) gene: a candidate tumour suppressor gene encoding a cell surface protein with similarity to neural cell adhesion molecules. *Cancer Surv* 1995; **24**: 3-17

3 **Christofori G**. Changing neighbours, changing behaviour: cell adhesion molecule-mediated signalling during tumour progression. *EMBO J* 2003; **22**: 2318-2323

4 **Cavallaro U**, Christofori G. Cell adhesion in tumor invasion and metastasis: loss of the glue is not enough. *Biochim Biophys Acta* 2001; **1552**: 39-45

5 **Rothbard JB**, Brackenbury R, Cunningham BA, Edelman GM. Differences in the carbohydrate structures of neural cell-adhesion molecules from adult and embryonic chicken brains. *J Biol Chem* 1982; **257**: 11064-11069

6 **Rutishauser U**, Acheson A, Hall AK, Mann DM, Sunshine J. The neural cell adhesion molecule (NCAM) as a regulator of cell-cell interactions. *Science* 1988; **240**: 53-57

7 **Huerta S**, Srivatsan ES, Venkatesan N, Peters J, Moatamed F, Renner S, Livingston EH. Alternative mRNA splicing in colon cancer causes loss of expression of neural cell adhesion molecule. *Surgery* 2001; **130**: 834-843

8 **Goridis C**, Brunet JF. NCAM: structural diversity, function and regulation of expression. *Semin Cell Biol* 1992; **3**: 189-197

9 **Hemperly JJ**, DeGuglielmo JK, Reid RA. Characterization of cDNA clones defining variant forms of human neural cell adhesion molecule N-CAM. *J Mol Neurosci* 1990; **2**: 71-78

10 **Pollerberg GE**, Burridge K, Krebs KE, Goodman SR, Schachner M. The 180-kD component of the neural cell adhesion molecule N-CAM is involved in a cell-cell contacts and cytoskeleton-membrane interactions. *Cell Tissue Res* 1987; **250**: 227-236

11 **Roesler J**, Srivatsan E, Moatamed F, Peters J, Livingston EH. Tumor suppressor activity of neural cell adhesion molecule in colon carcinoma. *Am J Surg* 1997; **174**: 251-257

12 **Greene FL**, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, Morrow M. AJCC Cancer Staging Manual, 6th ed. New York: Springer-Verlag, 2002: 113-125

13 **Koukoulis GK**, Patriarca C, Gould VE. Adhesion molecules and tumor metastasis. *Hum Pathol* 1998; **29**: 889-892

14 **Meyer T**, Hart IR. Mechanisms of tumour metastasis. *Eur J Cancer* 1998; **34**: 214-221

15 **Walsh FS**, Doherty P. Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. *Annu Rev Cell Dev Biol* 1997; **13**: 425-456

16 **Crossin KL**, Krushel LA. Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. *Dev Dyn* 2000; **218**: 260-279

17 **Panicker AK**, Buhusi M, Thelen K, Maness PF. Cellular signalling mechanisms of neural cell adhesion molecules. *Front Biosci* 2003; **8**: d900-d911

18 **Fogar P**, Basso D, Pasquali C, De Paoli M, Sperti C, Roveroni G, Pedrazzoli S, Plebani M. Neural cell adhesion molecule (N-CAM) in gastrointestinal neoplasias. *Anticancer Res* 1997; **17**: 1227-1230

19 **Tezel E**, Kawase Y, Takeda S, Oshima K, Nakao A. Expression of neural cell adhesion molecule in pancreatic cancer. *Pancreas* 2001; **22**: 122-125

20 **Roth J**, Zuber C, Wagner P, Blaha I, Bitter-Suermann D, Heitz PU. Presence of the long chain form of polysialic acid of the neural cell adhesion molecule in Wilms' tumor. Identification of a cell adhesion molecule as an oncodevelopmental antigen and implications for tumor histogenesis. *Am J Pathol* 1988; **133**: 227-240

21 **Hildebrandt H**, Becker C, Gluer S, Rosner H, Gerardy-Schahn R, Rahmann H. Polysialic acid on the neural cell adhesion molecule correlates with expression of polysialyltransferases and promotes neuroblastoma cell growth. *Cancer Res* 1998; **58**: 779-784

22 **Daniel L**, Trouillas J, Renaud W, Chevallier P, Gouvernet J, Rougon G, Figarella-Branger D. Polysialylated-neural cell adhesion molecule expression in rat pituitary transplantable tumors (spontaneous mammotropic transplantable tumor in Wistar-Furth rats) is related to growth rate and malignancy. *Cancer Res* 2000; **60**: 80-85

23 **Miyahara R**, Tanaka F, Nakagawa T, Matsuoka K, Isii K, Wada H. Expression of neural cell adhesion molecules (polysialylated form of neural cell adhesion molecule and L1-cell adhesion molecule) on resected small cell lung cancer specimens: in relation to proliferation state. *J Surg Oncol* 2001; **77**: 49-54

24 **Seki H**, Koyama K, Tanaka J, Sato Y, Umezawa A. Neural cell adhesion molecule and perineural invasion in gallbladder cancer. *J Surg Oncol* 1995; **58**: 97-100

25 **Seki H**, Tanaka J, Sato Y, Kato Y, Umezawa A, Koyama K. Neural cell adhesion molecule (NCAM) and perineural invasion in bile duct cancer. *J Surg Oncol* 1993; **53**: 78-83

26 **McLaughlin RB Jr**, Montone KT, Wall SJ, Chalian AA, Weinstein GS, Roberts SA, Wolf PF, Weber RS. Nerve cell adhesion molecule expression in squamous cell carcinoma of the head and neck: a predictor of propensity toward perineural spread. *Laryngoscope* 1999; **109**: 821-826

27 **Vural E**, Hutcheson J, Korourian S, Kechelava S, Hanna E. Correlation of neural cell adhesion molecules with perineural spread of squamous cell carcinoma of the head and neck. *Otolaryngol Head Neck Surg* 2000; **122**: 717-720

28 **Li R**, Wheeler T, Dai H, Ayala G. Neural cell adhesion molecule is upregulated in nerves with prostate cancer invasion. *Hum Pathol* 2003; **34**: 457-461

29 **Daniel L**, Durbec P, Gautherot E, Rouvier E, Rougon G, Figarella-Branger D. A nude mice model of human rhabdomyosarcoma lung metastases for evaluating the role of polysialic acids in the metastatic process. *Oncogene* 2001; **20**: 997-1004

30 **Fujimoto I**, Bruses JL, Rutishauser U. Regulation of cell adhesion by polysialic acid. Effects on cadherin, immunoglobulin cell adhesion molecule, and integrin function and independence from neural cell adhesion molecule binding or signaling activity. *J Biol Chem* 2001; **276**: 31745-31751

31 **Daniel L**, Bouvier C, Chetaille B, Gouvernet J, Luccioni A, Rossi D, Lechevallier E, Muracciole X, Coulange C, Figarella-Branger D. Neural cell adhesion molecule expression in renal cell carcinomas: relation to metastatic behavior. *Hum Pathol* 2003; **34**: 528-532

32 **Perl AK**, Dahl U, Wilgenbus P, Cremer H, Semb H, Christofori G. Reduced expression of neural cell adhesion molecule induces metastatic dissemination of pancreatic beta tumor cells. *Nat Med* 1999; **5**: 286-291

33 **Sasaki H**, Yoshida K, Ikeda E, Asou H, Inaba M, Otani M, Kawase T. Expression of the neural cell adhesion molecule in astrocytic tumors: an inverse correlation with malignancy. *Cancer* 1998; **82**: 1921-1931

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RAPID COMMUNICATION

Clinical significance of activity of ALT enzyme in patients with hepatitis C virus

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Abstract

AIM: To investigate serum alanine aminotransferase (ALT) levels in relation to the clinical, biochemical, ultrasonographic and histological characteristics of patients with hepatitis C virus.

METHODS: Duration of disease, HCV-RNA, liver steatosis, and the hepatitis activity index (HAI) were correlated with serum ALT in 36 patients with HCV. ALT values were also investigated in 16 control subjects without any liver diseases.

RESULTS: In bivariate analyses, ALT levels correlated with duration of HCV infection ($P < 0.01$), HCV-RNA ($P < 0.05$), and the HAI ($P < 0.01$). Among the components of the HAI, ALT concentrations were significantly associated with periportal bridging/necrosis ($P < 0.01$) and fibrosis ($P < 0.05$). In multivariate analysis, periportal bridging/necrosis ($\beta = 0.508$; $P < 0.01$), duration of HCV infection ($\beta = 0.413$; $P < 0.01$), and HCV-RNA ($\beta = 0.253$; $P < 0.05$) were independently associated with ALT activity. The normal ALT activity for men and women was < 23 IU/L and < 22 IU/L, respectively.

CONCLUSION: In patients with HCV, alterations in the liver tissue as reflected by ALT elevation are mainly associated with periportal bridging/necrosis, viral load and duration of disease. A cut-off value < 23 IU/L distinguished with high diagnostic accuracy healthy controls from patients with HCV.

Key words: Hepatitis C virus; Disease duration; Viral Load; Inflammation; Normal alanine aminotransferase

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INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease, frequently progressing to cirrhosis and increased risk of hepatocellular carcinoma[1-3]. Chronic hepatitis C is often silent, most of the times discovered only by routine serologic or biochemical testing[4-6]. Many attempts to identify the natural history and progression of hepatitis C infection have been made, but several aspects remain to be elucidated[7]. In individuals with chronic hepatitis C, viral load and elevated serum alanine aminotransferase (ALT) levels may have clinical relevance[8-10]. When parenchymal liver cells are damaged, aminotransferases leak from the liver into the blood, resulting in elevated levels of these enzymes in the bloodstream. The exact definition of the normal levels of serum ALT activity is crucial for screening and follow-up studies in hepatitis C infection[11,12]. It should be noted, however, that half of the untreated patients with chronic HCV infections display normal or minimally elevated serum ALT levels[13,14]. Accordingly, several studies have recently questioned whether previously established values to define normal ALT range are clinically accurate[11,12]. In this regard, it has been posited that the limits of normal for serum ALT should be revised accordingly[12].

In the present study, we sought to investigate serum ALT levels in relation to the clinical, biochemical, ultrasonographic and histological characteristics of patients with hepatitis C. We also aimed to study the normal level of ALT in Turkish healthy adults at low risk for chronic liver diseases. This information, in addition to daily clinical practice, may be clinically useful for research studies of hepatitis C and chronic liver diseases in Turkey.

MATERIALS AND METHODS

Study sample

A total of 36 patients (24 females, 12 males; mean age: 47.9 ± 13.2 years) with HCV infection were studied before the treatment with antiviral drugs. Patients with hemochromatosis, Wilson's disease, autoimmune hepatitis,

Table 1 General characteristics of the study patients with HCV infection

Characteristic	Entire cohort (n = 36)
Female, *n* (%)	24 (66.6%)
Age (yr)	47.9 ± 13.2
Estimated duration of HCV infection (mo)	58 (24-120)
HCV RNA (IU/mL)	2471075 ± 2490186
Body mass index (kg/m^2)	28.5 ± 3.9
Waist Circumference (cm)	98 ± 11
Fasting glucose (mg/dL)	96 ± 10
Haemoglobin (mg/dL)	13.9 ± 1.5
HOMA index	3.1 ± 2.4
Total cholesterol (mg/dL)	161 ± 36
HDL cholesterol (mg/dL)	48 ± 11
Triglycerides (mg/dL)	98 ± 41
Serum albumin (g/dL)	4.4 ± 0.3
Serum globulin (g/dL)	3.1 ± 0.6
Serum creatinine (mg/dL)	0.8 ± 0.2
AST (IU/L)	48 (32-67)
ALT (IU/L)	64 (34-80)
ALP (IU/L)	90 ± 33
GGT (IU/L)	45 (29-63)
LDH (IU/L)	184 ± 32
Total Bilirubin (mg/dL)	0.7 ± 0.3
Direct Bilirubin (mg/dL)	0.3 (0.2-0.4)
Platelet count (10^3/mm^3)	202 ± 54
Ferritin (ng/mL)	45 (34-77)
Positive ANA, *n* (%)	2 (5.5%)
Positive AMA, *n*	0
Positive ASMA, *n*	0
Positive LKM-1, *n*	0

Data expressed as means ± SD, or median (interquartile range), as appropriate; HOMA: Homeostasis model assessment; HDL: High-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutam.yl transpeptidase; LDH: Lactate dehydrogenase; ANA: Antinuclear antibodies; AMA: Antimitochondrial antibodies; ASMA: Anti-smooth muscle antibody; LKM-1: Liver-kidney microsomal antigen.

primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, alpha-1 antitrypsin deficiency, or malignancies were excluded from the present study. None of the subjects was using any medications, including estrogens, amiodarone, steroids, tamoxifen, or herbal supplements. Furthermore we excluded patients with daily alcohol intake exceeding 20 g/d. For control purposes, 16 healthy age- and gender-matched volunteers (9 females, 7 males) were recruited. All controls were judged to be in good health and confirmed as having normal liver by ultrasound. Subjects with a consumption of alcohol > 20 g/d or who were taking any medication were not included in the control group. All subjects underwent physical examination, anthropometric measurements and biochemical screening.

A written informed consent was obtained from all participants. Our study was in accordance with the ethical standards for human experimentation and approved by the Ethics Committee of the Uludag University Medical School.

Laboratory and virology assessment

Blood samples for the evaluation of alanine aminotransferase (ALT), and biochemical parameters were obtained after overnight fasting. Routine biochemical tests were carried out using commercially available kits (Hitachi, Tokyo, Japan). The HCV-RNA was determined in the sera using an RT-PCR assay (Amplicor, Roche, Mannheim), with a sensitivity of 70 copies/mL.

Ultrasound assessment

Liver ultrasound (US) scanning was performed to assess the degree of steatosis. All US procedures were performed by the same operator. Liver steatosis was assessed semi-quantitatively on a scale of 0 to 3:0, absent; 1, mild; 2, moderate and 3, severe.

Histological analysis

Ultrasonography-guided liver biopsies were performed under conscious sedation using a 16-gauge Klatskin needle. The length of histological specimens was not smaller than 2.5 cm. All biopsy specimens were placed in formalin solution for fixation and embedded in paraffin blocks. Serial sections (sectioned at 4 μm intervals) were stained with hematoxylin-eosin and Masson's trichrome. The hepatitis activity index (HAI), designed by Knodell and Desmet[15,16], was used to grade the severity of the necroinflammatory process and fibrosis. HAI comprises four separate scores, including periportal necrosis with or without bridging necrosis (0-10), intralobular degeneration and focal necrosis (0-4), portal inflammation (0-4) and fibrosis (0-4).

Statistical analysis

Variables are presented as counts and percentages, mean ± SD. Correlations among the study variables were assessed by means of the Pearson's correlation coefficients. Multivariate stepwise regression models were used to assess the independent predictors of ALT levels in patients with HCV infection. Cut-off values for serum ALT values were determined by means of the ROC curve analysis with the use of the MedCalc statistical software (Mariakerke, Belgium). A $P < 0.05$ was considered statistically significant. All computations were made using SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Bivariate analysis of serum ALT levels in patients with HCV infection

The characteristics of individuals with HCV infection are given in Table 1. The estimated median duration of HCV infection was 58 (interquartile range: 24-120) mo. Steatosis was present in 22 (61%) of the 36 HCV infected patients, of whom 8 (22%) had grade 1, 11 (30%) grade 2, and 3 (9%) grade 3. The histological findings of the study participants are shown in Table 2. In bivariate correlation analyses, ALT levels correlated with duration of HCV infection (r = 0.46, $P < 0.01$, Figure 1), HCV-RNA (r = -0.33, $P < 0.05$, Figure 2), and the HAI (r = 0.44, $P < 0.01$, Figure 3). Among the components of the HAI, ALT concentrations were significantly associated with periportal bridging/necrosis (r = 0.50, $P < 0.01$) and fibrosis (r = 0.37, $P < 0.05$).

Multivariate analysis

Multivariate stepwise regression analysis was used to

Table 2 Liver histology in the 36 study participants with HCV infection

Variable	Score
Hepatitis activity index	9.5 ± 3.7
Periportal necrosis with or without bridging necrosis	3.7 ± 2.2
Intralobular degeneration and focal necrosis	2.7 ± 1.1
Portal inflammation	3.0 ± 1.0
Fibrosis	1.9 ± 1.2

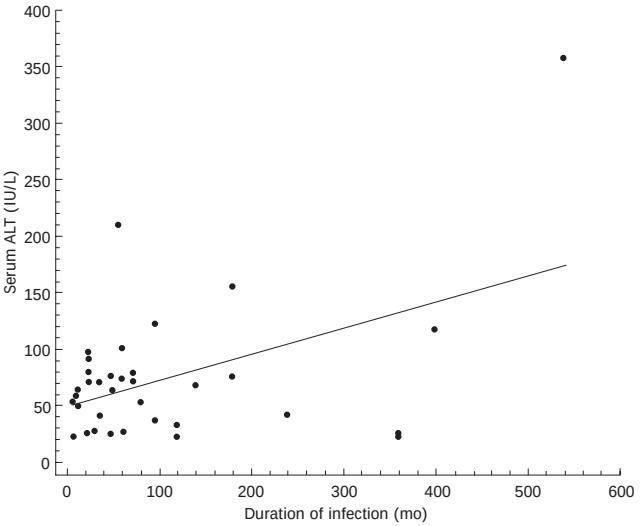

Figure 1 Scatter diagram and regression line showing a significant positive relationship between duration of HCV infection and serum alanine aminotransferase (r = 0.46, P < 0.01).

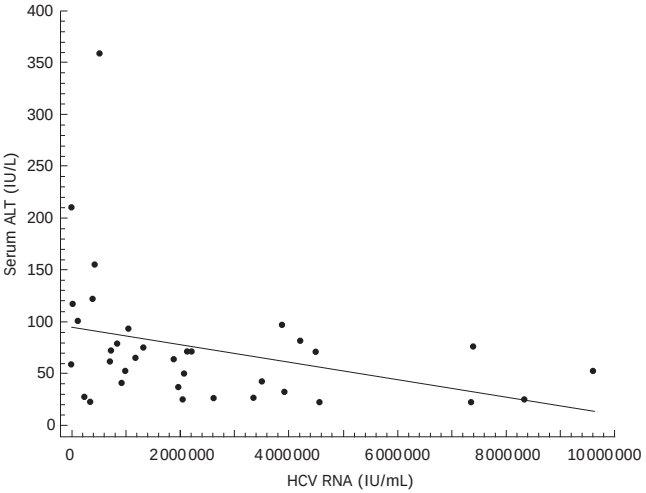

Figure 2 Scatter diagram and regression line showing a significant inverse relationship between viral load and serum alanine aminotransferase (r = -0.33, P < 0.05).

identify independent predictors of ALT levels in our patients with HCV infection. Serum ALT activity was considered as the dependent variable. All variables listed in Table 1 were entered into the multivariate model as independent variables. The results of this analysis showed that periportal bridging/necrosis (β = 0.508; P < 0.01), duration of HCV infection (β = 0.413; P < 0.01), and

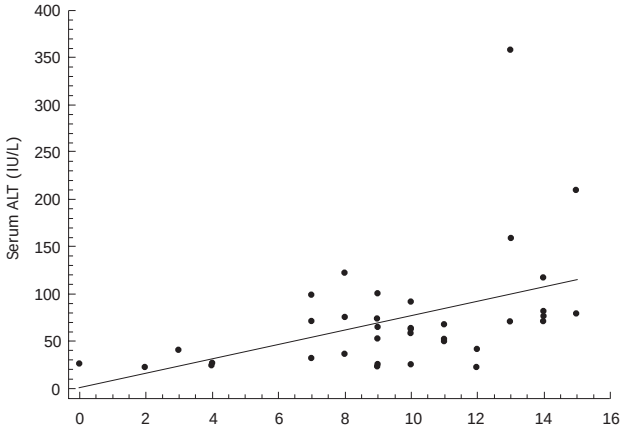

Figure 3 Scatter diagram and regression line showing a significant positive relationship between the Histology Activity Index and serum alanine aminotransferase (r = 0.44, P < 0.01).

HCV-RNA (β = 0.253; P < 0.05) were, in the order they entered into the model, independently associated with ALT levels.

Identification of a cut-off value for serum ALT levels according to gender

In order to establish a better cutoff value for ALT in hepatitis C screening in our Turkish population, the ALT levels were measured in 16 healthy age- and gender-matched volunteers. ALT levels in the control population were 18.2 ± 3.6 IU/L. The cutoff value was identified by construction of a ROC curve (receiver operating characteristic) in each gender. In females, the better cutoff value for ALT was at 22 IU/L, with sensitivity of 87.5% and specificity of 100% in identifying subjects without HCV infection (Figure 4). In males, the better cutoff value for ALT was at 22 IU/L, with sensitivity of 100% and specificity of 100% in identifying subjects without HCV infection (Figure 5).

DISCUSSION

This study provides insights into the correlates of ALT levels in the setting of patients with HCV infection. We found that, in our sample of Turkish patients, serum ALT levels were significantly and independently correlated with periportal bridging/necrosis, viral load and duration of HCV infection.

Serum ALT levels, as a measure of biochemical hepatitis activity, increased significantly with periportal bridging/necrosis, and this association was stronger than for other components of the HAI index. Our findings are in line with previous studies showing a statistically significant linear relationship between the degree of ALT elevation and the amount of liver injury based on the HAI score[17]. In our study, viral load was significantly and inversely correlated with mean ALT levels. This result is in keeping with the findings of Ito *et al*[18], who showed that mean viral load was significantly higher in chronic HCV patients with persistently normal ALT levels. In this regard, it has been hypothesized that immune response to HCV

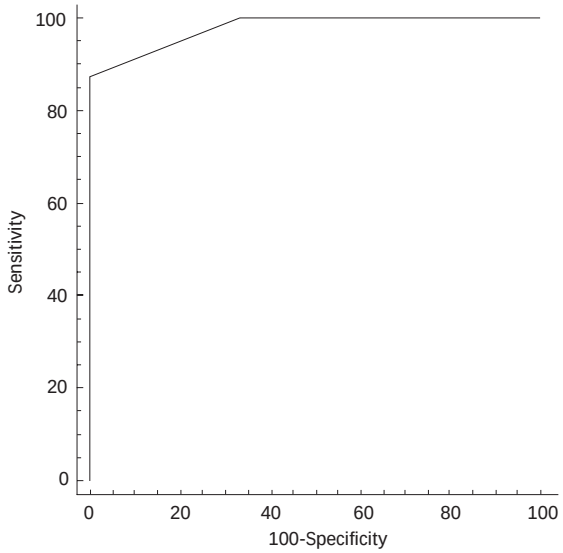

Figure 4 ROC curve of serum ALT for discriminating female HCV patients from women without liver disease. The better cutoff value for ALT was at 22 IU/L, with sensitivity of 87.5% and specificity of 100% in identifying subjects without HCV infection.

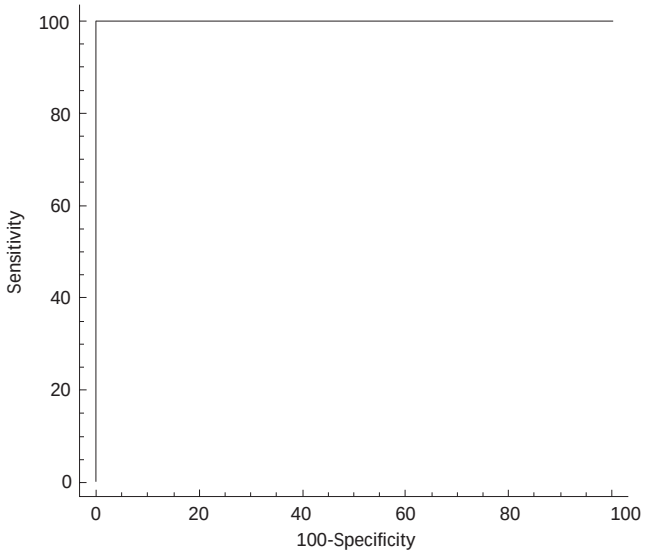

Figure 5 ROC curve of serum ALT for discriminating male HCV patients from men without liver disease. The better cutoff value for ALT was at 23 IU/L, with sensitivity of 100% and specificity of 100% in identifying subjects without HCV infection.

could play a role in rendering viral load smaller[18]. It should be noted, however, that some authors have reported higher ALT levels in patients with high viral load[19]. Another group has suggested that no significant difference in viral load exists between patients with abnormal ALT levels and those with normal ALT levels[20]. Although the reasons for conflicting data remain to be clarified, the discrepancies in the literature may be due at least in part to the presence of potential confounders such as ethnicity or different sample sizes. The duration of HCV infection may be of importance for the development of cirrhosis and patients with longer periods of infection may be more likely to have higher ALT levels[20]. In our study we found a positive association between ALT activity in the serum and duration of HCV infection. Some authors, however, have failed to demonstrate such a relationship[21]. In any case, it should be kept in mind that the onset of HCV infection may be difficult to establish in some patients, thereby rendering disease duration undefined.

Growing evidence has suggested that up to 25% of patients with chronic hepatitis C virus infection have persistently normal aminotransferase levels (10% to 40%, according to different studies)[22-24]. The normal range for ALT level was set in the 1950 s and has changed little since then. However, several recent studies have questioned whether previously established reference values to define normal ALT range are really accurate. Under these circumstances, it has been repeatedly suggested that the limits of normal ALT activity should be revised[25-27]. In most countries, the cutoff value for ALT is defined as twice the upper limit of the normal range of healthy individuals[28]. The normal ALT activity for men and women is < 23 IU/L and < 18 IU/L, respectively[28]. In order to gain more insights on the normal level of ALT in Turkish healthy adults at low risk for chronic liver diseases, we measured ALT activity in a control population from our country. By ROC curve analysis, we found that the optimal cutoff point in our population was 23 IU/L in males and 22 IU/L in females. Using our newly calculated cutoff value, we found that the sensitivity and specificity for detection of subjects without HCV infection were 100% and 100% in men and 87.5% and 100% in women, respectively. Hopefully, these new data obtained in the Turkish population should contribute to the ongoing discussion regarding new reference systems for the measurement of the catalytic activity of ALT in the clinical practice[29,30]. Given the small sample size, we believe that our findings may stimulate future studies on a larger number of patients.

In conclusion, we have provided evidence that the main correlates of ALT levels in HCV patients are periportal bridging/necrosis, viral load and duration of HCV infection. A cut-off value < 23 IU/L in males and 22 IU/L in females may distinguish with high diagnostic accuracy healthy control subjects from patients with HCV.

ACKNOWLEDGMENTS

The authors thank the assistance of the Research Fellows and the Scientific Staff at the Uludag University Medical School.

COMMENTS

Backgrounds

The exact definition of the normal levels of serum ALT activity is crucial for screening and follow-up studies in hepatitis C infection. However, half of the untreated patients with chronic HCV infections display normal or minimally elevated serum ALT levels. Accordingly, several studies have recently questioned whether previously established values to define normal ALT range are clinically accurate.

Research frontiers

Recent evidence has suggested that the limits of normal for serum ALT should be revised. In the present study, we sought to investigate serum ALT levels in relation to the clinical, biochemical, ultrasonographic and histological characteristics of patients with HCV.

Innovations and breakthroughs

We have provided evidence that the main correlates of ALT levels in HCV patients are periportal bridging/necrosis, viral load and duration of HCV infection.

Applications

A cut-off value < 23 IU/L in males and 22 IU/L in females may distinguish with high diagnostic accuracy healthy control subjects from patients with HCV.

Terminology

ALT elevation: aminotransferases leak from the liver into the blood when parenchymal liver cells are damaged.

Peer review

The study is of particular interest to the practical medicine. The results provide sufficient evidence that the activity of ALT correlates with histological changes, viral load and duration of infection in patients with HCV.

REFERENCES

1 **Hughes CA**, Shafran SD. Chronic hepatitis C virus management: 2000-2005 update. *Ann Pharmacother* 2006; **40**: 74-82
2 **Chevaliez S**, Pawlotsky JM. Hepatitis C virus: virology, diagnosis and management of antiviral therapy. *World J Gastroenterol* 2007; **13**: 2461-2466
3 **Chevaliez S**, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV-related liver disease. *Int J Med Sci* 2006; **3**: 35-40
4 **Sievert W**. Management issues in chronic viral hepatitis: hepatitis C. *J Gastroenterol Hepatol* 2002; **17**: 415-422
5 **Teoh NC**, Farrell GC. Management of chronic hepatitis C virus infection: a new era of disease control. *Intern Med J* 2004; **34**: 324-337
6 **Sherman M**. Chronic hepatitis C and screening for hepatocellular carcinoma. *Clin Liver Dis* 2006; **10**: 735-752
7 **Liu C**. Hepatitis C virus: virology and experimental systems. *Clin Liver Dis* 2006; **10**: 773-791
8 **Beld M**, Penning M, McMorrow M, Gorgels J, van den Hoek A, Goudsmit J. Different hepatitis C virus (HCV) RNA load profiles following seroconversion among injecting drug users without correlation with HCV genotype and serum alanine aminotransferase levels. *J Clin Microbiol* 1998; **36**: 872-877
9 **Mateescu RB**, Rimbas M, Staniceanu F, Zurac S, Dragomir P, Voiosu MR, Tataru M, Zota M, Bucurica S. Histological and nonhistological criteria in the evaluation of liver involvement in chronic hepatitis C. *Rom J Intern Med* 2006; **44**: 117-130
10 **Al-Quaiz MN**, Madani TA, Karawi MA. The natural history of hepatitis C virus infection. *Saudi Med J* 2003; **24** Suppl 2: S67-S70
11 **Mohamadnejad M**, Pourshams A, Malekzadeh R, Mohamadkhani A, Rajabiani A, Asgari AA, Alimohamadi SM, Razjooyan H, Mamar-Abadi M. Healthy ranges of serum alanine aminotransferase levels in Iranian blood donors. *World J Gastroenterol* 2003; **9**: 2322-2324
12 **Prati D**, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1-10
13 **Puoti C**, Castellacci R, Montagnese F. Hepatitis C virus carriers with persistently normal aminotransferase levels: healthy people or true patients? *Dig Liver Dis* 2000; **32**: 634-643
14 **Shiffman ML**, Diago M, Tran A, Pockros P, Reindollar R, Prati D, Rodriguez-Torres M, Lardelli P, Blotner S, Zeuzem S. Chronic hepatitis C in patients with persistently normal alanine transaminase levels. *Clin Gastroenterol Hepatol* 2006; **4**: 645-652
15 **Knodell RG**, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-435
16 **Desmet VJ**, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513-1520
17 **McCormick SE**, Goodman ZD, Maydonovitch CL, Sjogren MH. Evaluation of liver histology, ALT elevation, and HCV RNA titer in patients with chronic hepatitis C. *Am J Gastroenterol* 1996; **91**: 1516-1522
18 **Ito H**, Yoshioka K, Ukai K, Watanabe K, Yano M, Ishigami M, Mizutani T, Sasaki Y, Katano Y, Goto H. The fluctuations of viral load and serum alanine aminotransferase levels in chronic hepatitis C. *Hepatol Res* 2004; **30**: 11-17
19 **Hassan MI**, Kassim SK, Ali HS, Sayed el-DA, Khalifa A. Evaluation of nitric oxide (NO) levels in hepatitis C virus (HCV) infection: relationship to schistosomiasis and liver cirrhosis among Egyptian patients. *Dis Markers* 2002; **18**: 137-142
20 **Butt AA**, Tsevat J, Ahmad J, Shakil AO, Mrus JM. Biochemical and virologic parameters in patients co-infected with hepatitis C and HIV versus patients with hepatitis C mono-infection. *Am J Med Sci* 2007; **333**: 271-275
21 **Haydon GH**, Jarvis LM, Blair CS, Simmonds P, Harrison DJ, Simpson KJ, Hayes PC. Clinical significance of intrahepatic hepatitis C virus levels in patients with chronic HCV infection. *Gut* 1998; **42**: 570-575
22 **Gholson CF**, Morgan K, Catinis G, Favrot D, Taylor B, Gonzalez E, Balart L. Chronic hepatitis C with normal aminotransferase levels: a clinical histologic study. *Am J Gastroenterol* 1997; **92**: 1788-1792
23 **Persico M**, Perrotta S, Persico E, Terracciano L, Folgori A, Ruggeri L, Nicosia A, Vecchione R, Mura VL, Masarone M, Torella R. Hepatitis C virus carriers with persistently normal ALT levels: biological peculiarities and update of the natural history of liver disease at 10 years. *J Viral Hepat* 2006; **13**: 290-296
24 **Shiffman ML**, Diago M, Tran A, Pockros P, Reindollar R, Prati D, Rodriguez-Torres M, Lardelli P, Blotner S, Zeuzem S. Chronic hepatitis C in patients with persistently normal alanine transaminase levels. *Clin Gastroenterol Hepatol* 2006; **4**: 645-652
25 **Lozano M**, Cid J, Bedini JL, Mazzara R, Gimenez N, Mas E, Ballesta A, Ordinas A. Study of serum alanine-aminotransferase levels in blood donors in Spain. *Haematologica* 1998; **83**: 237-239
26 **Khedmat H**, Fallahian F, Abolghasemi H, Hajibeigi B, Attarchi Z, Alaeddini F, Holisaz MT, Pourali M, Sharifi S, Zarei N. Serum gamma-glutamyltransferase, alanine aminotransferase, and aspartate aminotransferase activity in Iranian healthy blood donor men. *World J Gastroenterol* 2007; **13**: 889-894
27 **Piton A**, Poynard T, Imbert-Bismut F, Khalil L, Delattre J, Pelissier E, Sansonetti N, Opolon P. Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. *Hepatology* 1998; **27**: 1213-1219
28 **Brinkmann T**, Dreier J, Diekmann J, Gotting C, Klauke R, Schumann G, Kleesiek K. Alanine aminotransferase cut-off values for blood donor screening using the new International Federation of Clinical Chemistry reference method at 37 degrees C. *Vox Sang* 2003; **85**: 159-164
29 **Tsai JF**, Jeng JE, Ho MS, Wang CS, Chang WY, Hsieh MY, Lin ZY, Tsai JH. Serum alanine aminotransferase level in relation to hepatitis B and C virus infections among blood donors. *Liver* 1997; **17**: 24-29
30 **Kariv R**, Leshno M, Beth-Or A, Strul H, Blendis L, Kokia E, Noff D, Zelber-Sagie S, Sheinberg B, Oren R, Halpern Z. Re-evaluation of serum alanine aminotransferase upper normal limit and its modulating factors in a large-scale population study. *Liver Int* 2006; **26**: 445-450

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RAPID COMMUNICATION

Risk factors of gastroesophageal reflux disease in Shiraz, southern Iran

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Abstract

AIM: To determine the prevalence and symptoms of gastroesophageal reflux disease (GERD) in a healthy general population in relation to demographic, lifestyle and health-seeking behaviors in Shiraz, southern Iran.

METHODS: A total of 1978 subjects aged > 35 years who referred to Gastroenterohepatology Research Center and who completed a questionnaire consisting of 27 questions for GERD in relation to demographic, lifestyle and health-seeking behaviors were included in this study for a period of five months. The validity and reliability of the questionnaire were determined.

RESULTS: The prevalence of GERD was 15.4%, which was higher in females (17.3%), in rural areas (19.8%), and in illiterate subjects (21.5%) and those with a mean age of 50.25 years. The prevalence was significantly lower in subjects having fried food (14.8%), and fruit and vegetables (14.6%). More symptoms were noticed in subjects consuming pickles (22.1%), taking aspirin (21%) and in subjects with psychological distresses (27.2%) and headaches (22%). The correlation was statistically significant between GERD and halitosis (18.3%), dyspepsia (30.6%), anxiety (19.5%), nightmares (23.9%) and restlessness (18.5%). Their health seeking behavior showed that there was a significant restriction of diet (20%), consumption of herbal medicine (19%), using over-the-counter drugs (29.9%) and consulting with physicians (24.8%). Presence of GERD symptoms was also significantly related to a previous family history of the disease (22.3%).

CONCLUSION: GERD is more common in females, rural and illiterate subjects and correlated with consumption of pickles, occurrence of headache, psychological distress, dyspepsia, halitosis, anxiety, nightmare and restlessness, and a family history of GERD and aspirin intake, but the correlation was negative with consumption of fat and fiber intake.

Key words: Reflux; Risk factors; Prevalence; Southern Iran

Saberi-Firoozi M, Khademolhosseini F, Yousefi M, Mehrabani D, Zare N, Heydari ST. Risk factors of gastroesophageal reflux disease in Shiraz, southern Iran. *World J Gastroenterol* 2007; 13(41): 5486-5491

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INTRODUCTION

Symptoms of gastroesophageal reflux disease (GERD) represent one of the most frequent health problems in the western world[1]. Approximately 10% of the American population suffer from daily heartburn and about one third have periodic symptoms[2]. Based on the population studied, the prevalence of the primary GERD symptoms, heartburn (a burning feeling behind the breast bone) or acid regurgitation (an acid taste in the mouth) varies between 9% and 42%[3]. The relationship between GERD and lifestyle habits, e.g. cigarette smoking, alcohol and coffee consumption, ingestion of medications such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), and diet has not been firmly established, and inconsistent results have been obtained from population-based studies[1,4-8].

As there are few population-based data on GERD in Asia[9-12], this study was performed for the first time in Shiraz, southern Iran with the aim of determining the prevalence of GERD symptoms and describing the demographic, lifestyle and health-seeking behaviors associated with GERD.

MATERIALS AND METHODS

Materials

This study was carried out in a group of GERD patients.

Table 1 Frequency of GERD symptoms and their correlation with different characteristics of subjects in Shiraz, Southern Iran (*n* = 1978)

Characteristics		GERD Symptoms (%)		*P* value
		Present	Absent	
Gender	Male	12.3	87.7	0.003
	Female	17.3	82.7	
Habitat	Urban	13	87	0.001
	Rural	19.8	80.2	
	Illiterate	21.5	78.5	
Education	Primary school	16.1	83.9	< 0.001
	High school	13.6	86.4	
	University	12.3	87.7	
Physical activity	No	14.8	85.2	0.373
	Yes	16.3	83.7	
Psychological distress	No	14.9	85.1	0.003
	Yes	27.2	72.8	
Recurrent headache	No	14.7	85.3	0.009
	Yes	22	78	
Past GI disease history	No	13.3	86.7	< 0.001
	Yes	22.3	77.7	
	Thin	10.5	89.5	
Body mass index	Normal	15.7	84.3	0.065
	Overweight	13.4	86.6	
	Obese	18.8	81.2	
Age (mean) (yr)		50.25	49.83	0.547

GERD: Gastroesophageal reflux disease.

Methods

In a population-based study, 3600 subjects were selected by cluster random sampling method based on postal code division of Shiraz, southern Iran into 17 districts. After clarifying the research project for each subject, he/she received an invitation letter to refer to Mottahari Digestive Clinic of Gastroenterohepatology Research Center affiliated to Shiraz University of Medical Sciences. The project was approved by the Ethics Committee of the university and a written consent was obtained from each patient participating in the study. The study was undertaken for a period of five months from April to September 2004 while 1978 subjects completed the questionnaire. The included subjects were aged > 35 years, of both genders, and from both urban and rural areas. A team of interviewers who had received an intense training completed the questionnaire consisting of 27 questions categorized into three sections of demographic, lifestyle and symptoms of GERD (Appendix 1). A gastroenterologist completed the clinical questions of the questionnaire in the clinic. The reliability and validity of the questionnaire were determined by requesting 100 subjects to be interviewed at our clinic by the same trained interviewers and a gastroenterologist for completion of the questionnaire, respectively. Heartburn was defined as a burning feeling in epigastric area that rises through the chest in substernal area and acid regurgitation as liquid coming back into the mouth leaving a bitter or sour taste. A subject was defined to suffer from GERD when he/she reported heartburn and/or acid regurgitation in the preceding year with a frequency of at least three times a week irrespective of its severity or duration. Sociodemographic variables included age, gender, habitat, marital status, educational level, biological characteristics, such as BMI [weight in kg in the fasting state divided by the square of the height in meters, resulting in five categories of thin (< 18 kg/m^2), normal (18-24.9 kg/m^2), overweight (25-29.9 kg/m^2), obese (30-40 kg/m^2) and very obese (> 40 kg/m^2)], lifestyle such as physical activity (at least 30 min/week or sufficient to produce adequate sweating), dietary habits, cigarette smoking, alcohol, coffee and tea consumption and the use of aspirin and NSAIDs. Rural and urban habitats were defined by the size of the residence area (under 30 000 inhabitants *vs* 30 000 inhabitants or more). Dyspepsia was defined as epigastric or upper abdominal symptoms (pain or discomfort) in the past year. Information was put directly into a computer database under supervision of a professional biostatician.

Statistical analysis

Statistical analysis was performed using the SPSS computer software package (Version 11.5, Chicago, Il). A *P* value of 0.05 or less was considered to be statistically significant and all reported *P* values were two sided using Chi-square tests.

RESULTS

Among 3600 visited households, the interview questionnaire was completed in 1978 subjects (response rate, 54.9%; mean age, 49.90 ± 11.14 years). Among the subjects, 29.4% were male, 56.6 % lived in urban and 43.4% in rural regions; 39.7%, 29.7%, 17.2% and 13.5% of the subjects were respectively in 35-44, 45-54, 55-64 and > 65 years age groups; 25.6%, 32.3%, 14.5% and 27.6% of the participants were illiterate, or with primary, high school and university educational levels, respectively. The reliability and validity of the questionnaire were 82% and 70%, respectively.

The prevalence rate of GERD was 15.4% (304 subjects, GERD occurring at least 3 times per week). Table 1 shows the prevalence rates of GERD in relation to demographic data, revealing that the prevalence was higher in females (17.3%, $P = 0.003$), in rural areas (19.8%, $P = 0.001$), and in illiterate subjects (21.5%, $P = 0.001$). In subjects with GERD, a higher prevalence of psychological distress (27.2%, $P = 0.003$) and headaches (22%, $P = 0.009$) was observed.

Table 2 demonstrates the frequency of GERD symptoms in relation to dietary, smoking and drinking habits and medication of the participants. The results indicated a lower prevalence in subjects having fried food (14.8%, $P = 0.005$), and a higher prevalence among those consuming pickles (22.1%, $P = 0.001$). There was no association between GERD symptoms and drinking spirits ($P = 0.095$) or water ($P = 0.063$) with meals, salt intake ($P = 0.458$) and physical activity ($P = 0.373$). The correlation between GERD symptoms and subjects being a current or former cigarette smoker (10.8%, $P = 0.055$) or water pipe smoker (18.7%, $P = 0.096$) was not significant either.

The prevalence of GERD was lower in subjects with more fruits and vegetables intake (14.6%, $P = 0.001$) and those drinking tea (14.9%, $P = 0.465$) and coffee (12.9%, $P = 0.701$), but was higher among those drinking alcohol (15.6%, $P = 0.205$) but the difference was significant from those with consumption of fruits and vegetables.

Table 2 Prevalence of GERD in relation to lifestyle of subjects in Shiraz, southern Iran (n = 1978)

Life style and dietary habits		GERD symptoms (%)		*P* value
		Present	Absent	
Pickle	Yes	22.1	77.9	< 0.001
	No	12.8	87.2	
Salt	No	15	85	0.458
	Yes	16.3	83.7	
Fried food	No	24	76	0.005
	Yes	14.8	85.2	
Fast food	No	15.7	84.3	0.518
	Yes	14.5	85.5	
Fiber	No	30.2	69.8	< 0.001
(fruit and vegetables)	Yes	14.6	85.4	
Cigarette	No	15.9	84.1	0.055
	Yes	10.8	89.2	
Water pipe	No	14.8	85.2	0.096
	Yes	18.7	81.3	
Tea	No	16.1	83.9	0.465
	Yes	14.9	85.1	
Coffee	No	15.4	84.6	0.701
	Yes	12.9	87.1	
Spirit with meal	No	16.7	83.3	0.095
	Yes	14	86	
Water with meal	No	17.6	82.4	0.063
	Yes	14.3	85.7	
Alcohol	No	9.7	90.3	0.205
	Yes	15.6	84.4	
Feeding duration (min)	< 10	16.5	83.5	
	10-20	14.5	85.5	0.519
	> 20	16.1	83.9	
Aspirin	No	14.7	85.3	0.02
	Yes	21	79	
NSAIDs	No	14.5	85.5	0.067
	Yes	17.9	82.1	

GERD: Gastroesophageal reflux disease; NSAIDs: Non-steroidal anti-inflammatory drugs.

We noticed more symptoms in subjects taking NSAIDs (17.9%, $P = 0.067$), and aspirin (21%, $P = 0.020$) (Table 2), but the difference was only significant for aspirin. Subjects with GERD symptoms are restricting their diets (20%, $P = 0.001$), taking herbal medicine (19.0%, $P = 0.001$), using the over-the-counter (OTC) drugs (29.9%, $P = 0.001$) and consulting with physicians (24.8%, $P = 0.001$). In subjects consuming medication advised by their friends, the difference was not statistically significant (23.5%, $P = 0.058$). Subjects with GERD had a significantly higher accurrence of halitosis (18.3%; $P = 0.024$), dyspepsia (30.6%; $P = 0.001$), anxiety (19.5%; $P = 0.001$), nightmare (23.9%; $P = 0.001$) and restlessness (18.5%; $P = 0.001$) (Table 3). There was an association between GERD symptoms and a family history of the disease (22.3%; P=0.001) (Table 1).

Table 3 Health-seeking behavior of subjects with GERD Symptoms in Shiraz, Southern Iran (n = 1978)

Health-seeking behavior and associated symptoms		GERD Symptoms (%)		*P* value
		Present	Absent	
Restricting diet	No	12.9	87.1	< 0.001
	Yes	20	80	
Herbal medicine intake	No	13.2	86.8	0.001
	Yes	19	81	
Medication advised	No	15.1	84.9	0.058
by friends	Yes	23.5	76.5	
Over-the-counter drugs	No	12.2	87.8	< 0.001
	Yes	29.9	70.1	
Visiting physician	No	10.5	89.5	< 0.001
	Yes	24.8	75.2	
Halitosis	No	14.2	85.8	0.024
	Yes	18.3	81.7	
Dyspepsia	No	8.9	91.1	< 0.001
	Yes	30.6	69.4	
Anxiety	No	9.5	90.5	< 0.001
	Yes	19.5	80.5	
Nightmare	No	12.1	87.9	< 0.001
	Yes	23.9	76.1	
Restlessness	No	10.2	89.8	< 0.001
	Yes	18.5	81.5	

DISCUSSION

It has been estimated that the digestive disease with the highest annual direct cost in the USA is GERD (about US$ 9.3 billion)[13]. Furthermore GERD patients have reported decrements in the health-related quality of life when compared with the general population[14,15]. Among western patients, heartburn and acid regurgitation are known to be specific for GERD[16].

In our population-based study, the prevalence of GERD was 15.4% defined as heartburn and/or acid regurgitation at least three times per week. In a population-based study, Khoshbaten[17] reported a prevalence of 2.7% for GERD as heartburn occurring at least thrice in recent two weeks in Tabriz, northwestern Iran. This difference may be due to his different case definition in the questionnaire. In a sample of general population in Germany, 18% of subjects suffered from GERD[18]. Wong *et al*[15] in a study by telephone contact reported a prevalence of 29.8% in a Chinese population. A study by telephone calls in a Spanish population showed a prevalence rate of 25.2%-34.7%[19]. A monthly prevalence of 1.6% was reported in Singapore[9]. Hu *et al*[20] demonstrated that only 5% of a Chinese population had GERD. In a large study of Taiwanese patients, 17% had at least one of three reflux symptoms daily[21]. Several factors that may influence the prevalence of GERD have been identified, including genetic factors and differences in body mass index and lifestyle[22-25]. Geographical differences in GERD prevalence are difficult to interpret due to the different case definitions and questionnaires used[4,26].

As shown in Table 1, the GERD prevalence was higher in females, rural areas, and illiterate subjects and those with a mean age of 50.25 years. Wong *et al*[11], Diaz-Rubio *et al*[12] and Mahadeva *et al*[19] also reported a higher prevalence of GERD in females. Some studies have not demonstrated a relationship between gender and GERD[10,27]. In relation to habitat, Diaz-Rubio *et al*[19] showed that GERD prevalence was higher in rural areas and in relation to educational level, a higher prevalence in illiterate subjects similar to our study. The relationship between a lower educational level and the frequency of GERD was described previously, which probably reflects the action of certain unhealthy lifestyle habits, or less ability to modify such habits[7,19].

In relation to life style, smoking and alcohol have often been cited as risk factors for GERD, although findings of

studies on this matter have been inconsistent[3,27,28,29]. According to Nocon *et al*[18], smoking was a risk factor for GERD, which was associated with reflux symptoms and was dose-dependent. Nilsson *et al*[3], reported that smoking and salt were risk factors for reflux symptoms. Our results showed no correlation between GERD and smoking.

In our study, we found no effect of alcohol, tea, coffee and spirits on GERD symptoms. In a population-based study, Nilsson *et al*[3] did not notice any effect for these risk factors. No relationship in Nocon *et al's* study[18] was found between the intake of alcohol and reflux symptoms. Wong *et al*[11] and Mahadeva *et al*[12] indicated the increase of GERD prevalence due to consumption of alcohol, which is not consistent with our results. Drinks such as tea and coffee have also been reported to be linked to GERD but this is controversial. Although tea has been shown to increase gastric acid secretion, it does not appear to contribute to GERD[22]. Wendle *et al*[30] showed that coffee increases GERD and the irritant effect of coffee was correlated to the caffeine content, but this has also been disputed. Chang *et al*[21] found no link between coffee or tea consumption and the incidence of GERD. Diaz-Rubio *et al*[19] also noticed that occurrence of GERD was inversely associated with coffee consumption. There was also no effect of tea or coffee on GERD symptoms in Nocon *et al's* study[18]. The inverse relationship with coffee, tea or alcohol consumption should not be interpreted as a protective role for these beverages. Restriction in drinking of tea, coffee and alcohol may arise from the suggestions of their friends, even in our country, where alcohol consumption is not legally allowed. The role of coffee as a promoter of gastroesophageal reflux disease[30] is also consistent, suggesting that the avoidance of coffee is a sound recommendation for GERD sufferers. In relation to fiber intake, El-Serag *et al*[5] and Nocon *et al*[18] reported that consumption of fruits were associated with GERD symptoms and found a protective effect of dietary fiber, which was similar to our results. In relation to dietary fat, El-Serag *et al*[5] found an increased risk of GERD in subjects with a high intake of dietary fat. The data of Nocon *et al*[18] also showed that subjects with reflux symptoms tend to have a diet richer in fat. It has been shown that dietary fat can increase the transient lower esophageal sphincter relaxation[31], possibly *via* release of cholecystokinin[32]. Therefore, the lower fat content in a population may explain, in part, the lower prevalence of GERD.

In relation to consumption of spirits, we found no association between reflux symptoms and consumption of spirits. These results were different from Nocon *et al*[18]. With regard to physical exercise, there are conflicting results[29]. According to Nocon *et al*[18], subjects with GERD were less active physically, but our data did not confirm these results. Some studies have observed an association between the use of aspirin or NSAIDs and the presence of GERD[19,33] and use of NSAIDs is a risk factor for erosive esophagitis[5,15,34], whereas others have not[27,35]. A higher consumption of NSAIDs and aspirin were visible in subjects of our study with GERD symptoms but was only statistically significant for aspirin. In relation to medical care utilization, the results vary among countries from 16% to 56%. A study from Singapore found that 40% of GERD sufferers used OTC drugs or visited a physician for GERD symptoms[9]. This is in contrast with a study in Minnesota, USA, in which only 5.4% of GERD sufferers visited physicians[27]. In the study of Wong *et al*[15], 48% of subjects with GERD had received treatment, 6% had taken OTC medication, and 35% had visited physicians. In our study, in relation to health seeking behavior, there were significant differences between GERD symptoms and restricting diets, consumption of herbal medicine, using OTC drugs and visiting a physician. Caution should be taken when applying the data to countries in which medical care is available on a fee-for-service basis. Patients usually associate certain nutritional habits with the occurrence of reflux symptoms, and the avoidance of certain food is often cited as a therapeutic measure[1,7,8]. Nevertheless, the causal role of particular food in the etiology of GERD is still unclear. A family history of reflux symptoms was reported as a risk factor for GERD[36]. GERD in the sufferer's spouse or a direct family member was reported to be associated with the presence of GERD[19]. These results were identical to our data. In relation to BMI, although most studies have confirmed the association between BMI and GERD symptoms, the results to date have remained inconsistent. Risk factors for GERD in the West have been shown to include a high BMI[27]. Similar to our study, a cohort study from New Zealand, also found no association between BMI and reflux symptoms[37]. In contrast, the large population-based HUNT 2 study reported an association between BMI and reflux symptoms. Nocon *et al*[18] reported similar results while being overweight or obese was significantly associated with GERD symptoms. Hampel *et al*[38] also found a significant association between obesity and GERD symptoms. The association between obesity and the prevalence and severity of GERD was confirmed by several other authors[5,39].

Our study showed that halitosis, headaches, psychological distress, anxiety, nightmares and restlessnes were common in GERD subjects. The importance of psychological distress was also suggested by others[8,19,40]. Some population surveys conducted in western countries have suggested that patients with GERD have a higher level of stress and anxiety[14,40,41]. Wong *et al*[15] showed that psychological morbidity may play an important role in health care-seeking behavior, and co-existing depression and anxiety may act as a catalyst for a patient to seek medical care, rather than as a cause of symptoms. Lower levels of psychological well-being were observed in subjects with GERD[42]. The important strength of our study was its large sample of subjects in a healthy population, which is representative of the adult population in our country between the ages of 35-75 years.

In conclusion, the prevalence of GERD (15.4%) was significantly higher in females, rural and illiterate subjects. An inverse correlation was seen between GERD and consumption of fat and fiber intake. A correlation was noticed between GERD and pickle consumption, occurrence of headache, psychological distress, dyspepsia, halitosis, anxiety, nightmare and restlessness and a pervious family history of GERD. The association between GERD and aspirin was also significant. Future longitudinal studies and follow-ups are needed to clarify other possible risk factors and associations with GERD.

COMMENTS

Background

Symptoms of gastroesophageal reflux disease (GERD) represent one of the most frequent health problems in the western world. When compared with the general population, GERD patients have reported decrements in the health-related quality of life. Based on the population studied, the prevalence of the primary GERD symptoms, heartburn or acid regurgitation varies between 9% and 42%. The relationship between GERD and lifestyle habits, e.g. cigarette smoking, alcohol and coffee consumption, ingestion of medications such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), and diet has not been firmly established, and inconsistent results have been obtained by population-based studies.

Research frontiers

The study was performed to determine the relationship between GERD and demographic factors, lifestyle habits, family history, health-seeking behaviors and other GI symptoms. How GERD might affect quality of life awaits further studies.

Innovations and breakthroughs

Many other studies on GERD were conducted by telephone surveys or in the form of questionnaires. In our study, however, subjects were interviewed face-to-face by a team of trained interviewers using a questionnaire for which validity and reliability had been determined. Both rural and urban inhabitants participated in this study, making possible a comparison of these two populations in regards to GERD. In our population-based study, the prevalence of GERD was 15.4%, which is higher than that reported by some other studies in Iran. Obesity, consumption of spirits and smoking have been suggested as the most important lifestyle risk factors for GERD symptoms, but we found no association between reflux symptoms and consumption of spirits, smoking or BMI.

Applications

The findings of this study are helpful to both the clinicians in handling GERD patients and patients in primary and secondary healthcare.

Terminology

Heartburn is defined as a burning pain or discomfort behind the breast bone. Acid regurgitation is referred to liquid coming back into the mouth leaving a bitter or sour taste.GERD, in this study, is considered as heartburn and/or acid regurgitation occurring at least three times per week.

Peer review

This is an extensive cross sectional study performed in Iran and gives attention to the contributing factors and demographics of GERD in the country of the authors.

REFERENCES

1 **Dent J**, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2005; **54**: 710-717
2 **Nebel OT**, Fornes MF, Castell DO. Symptomatic gastroesophageal reflux: incidence and precipitating factors. *Am J Dig Dis* 1976; **21**: 953-956
3 **Nilsson M**, Johnsen R, Ye W, Hveem K, Lagergren J. Prevalence of gastro-oesophageal reflux symptoms and the influence of age and sex. *Scand J Gastroenterol* 2004; **39**: 1040-1045
4 **Stanghellini V**. Three-month prevalence rates of gastrointestinal symptoms and the influence of demographic factors: results from the Domestic/International Gastroenterology Surveillance Study (DIGEST). *Scand J Gastroenterol Suppl* 1999; **231**: 20-28
5 **El-Serag HB**, Johanson JF. Risk factors for the severity of erosive esophagitis in Helicobacter pylori-negative patients with gastroesophageal reflux disease. *Scand J Gastroenterol* 2002; **37**: 899-904
6 **Labenz J**, Jaspersen D, Kulig M, Leodolter A, Lind T, Meyer-Sabellek W, Stolte M, Vieth M, Willich S, Malfertheiner P. Risk factors for erosive esophagitis: a multivariate analysis based on the ProGERD study initiative. *Am J Gastroenterol* 2004; **99**: 1652-1656
7 **Oliveria SA**, Christos PJ, Talley NJ, Dannenberg AJ. Heartburn risk factors, knowledge, and prevention strategies: a population-based survey of individuals with heartburn. *Arch Intern Med* 1999; **159**: 1592-1598
8 **Bolin TD**, Korman MG, Hansky J, Stanton R. Heartburn: community perceptions. *J Gastroenterol Hepatol* 2000; **15**: 35-39
9 **Ho KY, Kang JY**, Seow A. Prevalence of gastrointestinal symptoms in a multiracial Asian population, with particular reference to reflux-type symptoms. *Am J Gastroenterol* 1998; **93**: 1816-1822
10 **Goh KL**, Chang CS, Fock KM, Ke M, Park HJ, Lam SK. Gastro-oesophageal reflux disease in Asia. *J Gastroenterol Hepatol* 2000; **15**: 230-238
11 **Wong WM**, Lam KF, Lai KC, Hui WM, Hu WH, Lam CL, Wong NY, Xia HH, Huang JQ, Chan AO, Lam SK, Wong BC. A validated symptoms questionnaire (Chinese GERDQ) for the diagnosis of gastro-oesophageal reflux disease in the Chinese population. *Aliment Pharmacol Ther* 2003; **17**: 1407-1413
12 **Mahadeva S**, Raman MC, Ford AC, Follows M, Axon AT, Goh KL, Moayyedi P. Gastro-oesophageal reflux is more prevalent in Western dyspeptics: a prospective comparison of British and South-East Asian patients with dyspepsia. *Aliment Pharmacol Ther* 2005; **21**: 1483-1490
13 **Sandler RS**, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, Shah S, Avdic A, Rubin R. The burden of selected digestive diseases in the United States. *Gastroenterology* 2002; **122**: 1500-1511
14 **Frank L**, Kleinman L, Ganoczy D, McQuaid K, Sloan S, Eggleston A, Tougas G, Farup C. Upper gastrointestinal symptoms in North America: prevalence and relationship to healthcare utilization and quality of life. *Dig Dis Sci* 2000; **45**: 809-818
15 **Wong WM**, Lai KC, Lam KF, Hui WM, Hu WH, Lam CL, Xia HH, Huang JQ, Chan CK, Lam SK, Wong BC. Prevalence, clinical spectrum and health care utilization of gastro-oesophageal reflux disease in a Chinese population: a population-based study. *Aliment Pharmacol Ther* 2003; **18**: 595-604
16 **Klauser AG**, Schindlbeck NE, Muller-Lissner SA. Symptoms in gastro-oesophageal reflux disease. *Lancet* 1990; **335**: 205-208
17 **Khoshbaten M**. Gastro-esophageal reflux disease in northwestern Tabriz, Iran. *Indian J Gastroenterol* 2003; **22**: 138-139
18 **Nocon M**, Labenz J, Willich SN. Lifestyle factors and symptoms of gastro-oesophageal reflux-a population-based study. *Aliment Pharmacol Ther* 2006; **23**: 169-174
19 **Diaz-Rubio M**, Moreno-Elola-Olaso C, Rey E, Locke GR 3rd, Rodriguez-Artalejo F. Symptoms of gastro-oesophageal reflux: prevalence, severity, duration and associated factors in a Spanish population. *Aliment Pharmacol Ther* 2004; **19**: 95-105
20 **Hu WHC**, Hui WM, Lam CLK, Lam SK. Anxiety and depression are co–factors determining health care utilization in patients with dyspepsia: A Hong Kong population-based study. *Gastroenterology* 1997; **112** Suppl 1: A153
21 **Chang CS**, Poon SK, Lien HC, Chen GH. The incidence of reflux esophagitis among the Chinese. *Am J Gastroenterol* 1997; **92**: 668-671
22 **Dubey P**, Sundram KR, Nundy S. Effect of tea on gastric acid secretion. *Dig Dis Sci* 1984; **29**: 202-206
23 **Kahrilas PJ**, Gupta RR. The effect of cigarette smoking on salivation and esophageal acid clearance. *J Lab Clin Med* 1989; **114**: 431-438
24 **Ireland A**, Lyrenas E, Tippett M. Provocation of transient lower Esophageal sphincter relaxations and gastroesophageal reflux by intraduodenal fat. *Gastroenterology* 1990; **98**: A361
25 **Feldman M**, Barnett C. Relationships between the acidity and osmolality of popular beverages and reported postprandial heartburn. *Gastroenterology* 1995; **108**: 125-131
26 **Louis E**, DeLooze D, Deprez P, Hiele M, Urbain D, Pelckmans P, Deviere J, Deltenre M. Heartburn in Belgium: prevalence, impact on daily life, and utilization of medical resources. *Eur J Gastroenterol Hepatol* 2002; **14**: 279-284
27 **Locke GR 3rd**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ

3rd. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456
28 **Meining A**, Classen M. The role of diet and lifestyle measures in the pathogenesis and treatment of gastroesophageal reflux disease. *Am J Gastroenterol* 2000; **95**: 2692-2697
29 **Nandurkar S**, Locke GR 3rd, Fett S, Zinsmeister AR, Cameron AJ, Talley NJ. Relationship between body mass index, diet, exercise and gastro-oesophageal reflux symptoms in a community. *Aliment Pharmacol Ther* 2004; **20**: 497-505
30 **Wendl B**, Pfeiffer A, Pehl C, Schmidt T, Kaess H. Effect of decaffeination of coffee or tea on gastro-oesophageal reflux. *Aliment Pharmacol Ther* 1994; **8**: 283-287
31 **Nebel OT**, Castell DO. Lower esophageal sphincter pressure changes after food ingestion. *Gastroenterology* 1972; **63**: 778-783
32 **Ledeboer M**, Masclee AA, Batstra MR, Jansen JB, Lamers CB. Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. *Gut* 1995; **36**: 39-44
33 **Kotzan J**, Wade W, Yu HH. Assessing NSAID prescription use as a predisposing factor for gastroesophageal reflux disease in a Medicaid population. *Pharm Res* 2001; **18**: 1367-1372
34 **Newton M**, Kamm MA, Quigley T, Burnham WR. Symptomatic gastroesophageal reflux in acutely hospitalized patients. *Dig Dis Sci* 1999; **44**: 140-148
35 **Haque M**, Wyeth JW, Stace NH, Talley NJ, Green R. Prevalence, severity and associated features of gastro-oesophageal reflux and dyspepsia: a population-based study. *N Z Med J* 2000; **113**: 178-181
36 **Wright RA**, Hurwitz AL. Relationship of hiatal hernia to endoscopically proved reflux esophagitis. *Dig Dis Sci* 1979; **24**: 311-313
37 **Talley NJ**, Weaver AL, Zinsmeister AR, Melton LJ 3rd. Onset and disappearance of gastrointestinal symptoms and functional gastrointestinal disorders. *Am J Epidemiol* 1992; **136**: 165-177
38 **Hampel H**, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. *Ann Intern Med* 2005; **143**: 199-211
39 **Ruhl CE**, Everhart JE. Overweight, but not high dietary fat intake, increases risk of gastroesophageal reflux disease hospitalization: the NHANES I Epidemiologic Followup Study. First National Health and Nutrition Examination Survey. *Ann Epidemiol* 1999; **9**: 424-435
30 **Johnston BT**, Gunning J, Lewis SA. Health care seeking by heartburn sufferers is associated with psychosocial factors. *Am J Gastroenterol* 1996; **91**: 2500-2504
41 **Koloski NA**, Talley NJ, Boyce PM. Epidemiology and health care seeking in the functional GI disorders: a population-based study. *Am J Gastroenterol* 2002; **97**: 2290-2299
42 **Dimenas E**. Methodological aspects of evaluation of Quality of Life in upper gastrointestinal diseases. *Scand J Gastroenterol Suppl* 1993; **199**: 18-21

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Appendix 1

Questionnaire
Gastroenterohepatology Research Center, Shiraz University of Medical Sciences
Frequency and associated factors of digestive and hepatic disorders in subjects aged ≥ 35 yr in Shiraz, Southern Iran

Questionnaire No: …….	Date: ……
Sex	Female □ Male □
Age	.....years
Marital status	Single □ Married □ Widow □ Divorced □
Habitat	Urban □ Rural □
Family size	.....
Education	Illiterate □ Primary □ Middle □ High school □ University □
Occupation	.....
Past medical history	Headache □ Psychological distress □ Hyperlipidemia □
Physical activity	.....Times per week
Family history of gastrointestinal diseases	Yes □ No □ If Yes : Specify the disease
Number of meals/day	Breakfast □ Lunch □ Dinner □ More □
Duration of serving each meal	.....min
Pickles consumption with meal?	Yes □ No □
Salt consumption with meal?	Yes □ No □
Having fast food?	Yes □ No □
If yes, how many/week?	.....
Having fried foods?	Yes □ No □
Any smoking?	Yes □ (Cigarette □ Water pipe □) No □
If yes	...../day .....year
Type of analgesics regularly used?	NSAIDs □ Aspirin □
Having fibers (fruits,vegetables)	Yes □ No □
Type and time of drinks?	Tea, after meal □ Water, with or after meal □ Coffee, after meal □ Spirit, with or after meal □
Alcohol drinking?	Yes, usually □ Yes, occasionally □ Never □
Any history of Gastroesophageal reflux (Heartburn or acid regurgitation) during last year?	Yes □ No □
Any upper abdominal discomfort or dyspepsia?	Yes □ No □
Health care-seeking behavior?	Diet restriction □ Herbal medicine□ Using medicine suggested by friends □ Over-the-counter drugs □ Visiting a physician □
Any complaints of:	Anxiety □ Nightmares □ Restlessness □ Halitosis □
Name and Signature of interviewer	……

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RAPID COMMUNICATION

A low prevalence of *H pylori* and endoscopic findings in HIV-positive Chinese patients with gastrointestinal symptoms

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Abstract

AIM: To compare the prevalence of *H pylori* infection, peptic ulcer, cytomegalovirus (CMV) infection and Candida esophagitis in human immunodeficiency virus (HIV)-positive and HIV-negative patients, and evaluate the impact of CD4 lymphocyte on *H pylori* and opportunistic infections.

METHODS: A total of 151 patients (122 HIV-positive and 29 HIV-negative) with gastrointestinal symptoms were examined by upper endoscopy and biopsy. Samples were assessed to determine the prevalence of *H pylori* infection, CMV, candida esophagitis and histologic chronic gastritis.

RESULTS: The prevalence of *H pylori* was less common in HIV-positive patients (22.1%) than in HIV-negative controls (44.8%; $P < 0.05$), and the prevalence of *H pylori* displayed a direct correlation with CD4 count stratification in HIV-positive patients. In comparison with HIV-negative group, HIV-positive patients had a lower incidence of peptic ulcer (20.7% *vs* 4.1%; $P < 0.01$), but a higher prevalence of chronic atrophy gastritis (6.9% *vs* 24.6%; $P < 0.05$), Candida esophagitis and CMV infection. Unlike HIV-negative group, *H pylori* infection had a close relationship to chronic active gastritis ($P < 0.05$). In HIV-positive patients, chronic active gastritis was not significantly different between those with *H pylori* infection and those without.

CONCLUSION: The lower prevalence of *H pylori* infection and peptic ulcer in HIV-positive patients with gastrointestinal symptoms suggests a different mechanism of peptic ulcerogenesis and a different role of *H pylori* infection in chronic active gastritis and peptic ulcer. The pathogen of chronic active gastritis in HIV-positive patients may be different from the general population that is closely related to *H pylori* infection.

Key words: Human immunodeficiency virus; Endoscopy; Cytomegalovirus; Candida esophagitis; *H pylori*; Peptic ulcer; Chronic gastritis

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INTRODUCTION

H pylori has been extensively studied and proven to be the main cause of chronic gastritis and peptic ulcer in the HIV-negative population[1,2]. The reported prevalence of *H pylori* in unselected populations ranges from 32% to 65%[3-6]. Over 90% patients with chronic active gastritis showed an evidence of *H pylori* infection[3,4,7], and 70%-100% of those patients had peptic ulcer disease[1,5,7].

In contrast, the prevalence of *H pylori* infection in patients infected with HIV has been reported to be remarkably low[8-11]. Reasons for these lower rates of *H pylori* infection remain unclear. Other studies showed that *H pylori* infection is similar in both HIV-positive and HIV-negative patients[12,13]. Patients infected with HIV, with or without acquired immune deficiency syndrome (AIDS), have a high incidence (50%-90%) of upper gastrointestinal symptoms[14]. The immune deficiencies caused by HIV give rise to many different gastrointestinal opportunistic infections, such as cytomegalovirus (CMV) infection and fungal esophagitis[15,16].

The aims of our study are to assess the prevalence of *H pylori* infection and the association with histological chronic active gastritis in HIV-positive patients with gastrointestinal symptoms. The impact of $CD4^+$ count on the prevalence of *H pylori*, gastric CMV infection and Candida esophagitis was also evaluated.

MATERIALS AND METHODS

Patients

The study was carried out at Beijing You'an Hospital,

Capital Medical University, Beijing, the largest referral center for management of HIV infection and HIV-related complications in China, from January 2003 to March 2006. Endoscopy was performed in 151 patients for gastrointestinal symptoms such as abdominal pain, dyspepsia, diarrhea, nausea, vomiting, gastrointestinal bleeding, and odynophagia or dysphagia.

The study groups consisted of 122 HIV-positive patients (49 males and 73 females; mean age 40.8 ± 7.9, range 26-60 years) and 29 age-matched HIV-negative patients (15 males and 14 females; mean age 49.5 ± 12.7, range 28-77 years) as control groups. The absolute $CD4^+$ lymphocyte count of HIV-positive patients at the time of endoscopic examination was measured with FACS Count Reagents (BD Company, USA). Patients all gave their consent before undergoing endoscopy, and the symptoms, consumption of medications within one month, including antibiotics, proton pump inhibitors were also recorded.

Endoscopy, diagnosis and histology

Video-endoscopes (Olympus XQ240, Tokyo, Japan) were used for the procedure. All patients underwent three biopsies from the lesser and greater curvature of the gastric antrum and lesser curvature of lower body, one for Rapid Urease Test (RUT) and two for histology. Additional biopsies were obtained from endoscopic lesions such as ulceration. The biopsy specimens were placed in 10% formaldehyde at the time of endoscopy and stained with hematoxylin-eosin, Warthin-Starry stains for histologic chronic gastritis and *H pylori* infection, and immunocytochemical techniques were performed for CMV infection (Monoclonal Mouse Anti-Human Cytomegalovirus, Dako). The *H pylori* infection was diagnosed by positive identification of both the organism on histology (Warthin-Starry) and RUT. The histologic gastritis was diagnosed according to the Sydney criteria[17]. Specimens were reviewed by only one pathologist who was blind to the status of those patients in present study.

The Candida esophagitis was diagnosed by sheathed brush cytology from endoscopic lesions, and gross appearance of mucosal presented with white plaques. Specimens obtained by sheathed brush should be smeared onto slides for fungi.

Statistical analysis

Chi-square test or Fisher exact probability tests were used to compare the prevalence of *H pylori*, CMV infection, Candida esophagitis, and peptic ulcer between HIV-positive patients, control groups, and HIV-infected patients with higher and lower $CD4^+$ counts and the use of antibiotics and proton pump inhibitors. Independent sample *t* test was used to compare the age and sex between the HIV-positive and control groups. A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

The patient data and the prevalence of *H pylori* and endoscopic findings in HIV-positive patients and HIV-negative patients are shown in Table 1. The gastrointestinal symptoms of HIV-positive patients were mostly nonspecific, such as diarrhea, dyspepsia, abdominal pain, nausea, vomiting, and odynophagia or dysphagia. Only the occurrence of symptoms of diarrhea, odynophagia, and dysphagia in HIV-positive patients was significantly higher than that of control group ($P < 0.05$). The prevalence of *H pylori* infection was significantly lower in the HIV-positive group than that of HIV-negative control group (27/122; 22.1% *vs* 13/29; 44.8%, $P < 0.05$). Endoscopic examination revealed more patients with peptic ulcer in HIV-negative group than in HIV-positive group (6/29; 20.7% *vs* 5/122; 4.1%, $P < 0.01$). More histologic chronic atrophy gastritis was found in HIV-positive patients than in HIV-negative group (30/122; 24.6% *vs* 2/29; 6.9%, $P < 0.05$). Opportunistic infection by CMV was noted in 4.9% (6/122) HIV-positive patients but none in the HIV-negative group ($P = 0.49$). The incidence of Candida esophagitis in HIV-positive patients (19/122; 15.6%) was significantly higher than that of HIV-negative patients ($P < 0.05$).

H pylori infection was less common in those with $CD4^+$ counts < 200/μL than those with $CD4^+$ counts > 200/μL in HIV-positive patients (8/57; 14.0% *vs* 19/65; 29.2%, $P < 0.05$). Interestingly, the prevalence of *H Pylori* infection displayed a direct correlation with the $CD4^+$ lymphocyte count stratification in HIV-positive patients (Table 2). The Candida esophagitis was significantly more common in

Table 1 Patient data and clinicopathology

	HIV-positive (*n* = 122) (%)	HIV-negative (*n* = 29) (%)	*P*
Age (yr)	40.8 ± 7.9	49.5 ± 12.7	NS
Male	49	15	NS
Female	73	14	NS
Gastrointestinal symptoms			NS
Abdominal pain and distention	38	14	NS
Dyspepsia	42	6	NS
Diarrhea	27	1	0.02
Nausea and vomiting	47	8	NS
Odynophagia and dysphagia	21	0	0.035
Others	16	6	NS
Consumption of medications within one month, *n* (%)			
Antibiotics	47 (38.5)	3 (10.3)	0.004
Proton pump inhibitor	3 (2.5)	4 (13.8)	0.034
H pylori infection	27 (22.1)	13 (44.8)	0.013
Candida esophagitis	19 (15.6)	0 (0)	0.05
Peptic ulcer	5 (4.1)	6 (20.7)	0.007
Chronic atrophy gastritis	30 (24.6)	2 (6.9)	0.036
CMV infection	6 (4.9)	0 (0)	0.49

CMV: Cytomegalovirus; NS: Not significant.

Table 2 *H pylori* infection and previous use of antibiotics related to $CD4^+$ count in HIV-positive patients *n* (%)

$CD4^+$ count	*H pylori* infection	*P*	Antibiotic therapy	*P*
$CD4^+ \geq 200/\mu L$ (*n* = 65)	19 (29.2)	0.044	19 (29.3)	0.024
$CD4^+ < 200/\mu L$ (*n* = 57)	8 (14.0)		28 (49.1)	
$CD4^+ \geq 100/\mu L$ (*n* = 85)	24 (28.2)	0.014	29 (34.1)	0.13
$CD4^+ < 100/\mu L$ (*n* = 37)	3 (8.1)		18 (48.6)	

Table 3 Relationship of $CD4^+$ count to *H pylori* infection and Endoscopic Findings in HIV-positive patients *n* (%)

	$CD4^+ \geqslant 200/\mu L$ (*n* = 65)	$CD4^+ < 200/\mu L$ (*n* = 57)	*P*
H pylori infection	19 (29.2)	8 (14)	0.044
Candida esophagitis	4 (6.2)	15 (26.3)	0.002
Peptic ulcer	2 (3.1)	3 (5.3)	0.881
Chronic atrophy gastritis	13 (20)	17 (29.8)	0.209
CMV infection	1 (1.5)	5 (8.8)	0.155

Table 4 Relationship of chronic active gastritis to *H pylori* infection

	HIV-positive group (*n* = 122)		HIV-negative group (*n* = 29)	
	H pylori $^+$ (*n* = 27)	*H pylori* $^-$ (*n* = 95)	*H pylori* $^+$ (*n* = 13)	*H pylori* $^-$ (*n* = 16)
Chronic active gastritis, *n* (%)	8 (29.6)	16 (16.8)	8 (61.5)	1 (6.3)
P	0.140		0.005	

HIV-positive patients with $CD4^+$ count < 200/μL than those with $CD4^+$ count > 200/μL (15/57; 26.3% *vs* 4/65; 6.2%, $P < 0.01$), and the average $CD4^+$ counts of patients with Candida esophagitis was 116.47 ± 133.08/μL. The CMV infection was more common in HIV-positive patients with $CD4^+$ count < 200/μL than those with $CD4^+$ count > 200/μL , but it was not statistically significant (5/57; 8.8% *vs* 1/65; 1.5%, $P = 0.155$) (Table 3).

Histological examination revealed less chronic active gastritis in HIV-positive patients than in HIV-negative control group (24/122; 19.7% *vs* 9/29; 31%, *P* = NS), but the difference was not statistically significant. The relationship of *H pylori* infection with chronic active gastritis was evaluated in HIV-positive and HIV-negative patients (Table 4). In HIV-negative group, the incidence of chronic active gastritis was significantly higher in those with *H pylori* infection (61.5%) than those without (6.3%; $P < 0.01$). In HIV-positive group, the rate of chronic active gastritis was not significantly different between those with *H pylori* infection (29.6%) and those without (16.8%; $P = 0.14$).

The relationship in *H pylori* and CMV infection and peptic ulcer was evaluated between the two groups of patients. Peptic ulcer was detected in five HIV-positive patients, one of whom (20%) was positive for *H pylori* infection and one (20%) for CMV infection. In HIV-negative group, six patients were diagnosed as having peptic ulcer, four (67%) as *H pylori* infection and none as CMV infection.

The previous use of antibiotics and proton pump inhibitor was also evaluated between HIV-positive and HIV-negative patients (47/122; 38.5% *vs* 3/29; 10.3%, $P < 0.01$ and 3/122; 2.5% *vs* 4/29; 13.8%, $P < 0.05$, respectively) (Table 1). If $CD4^+$ count was taken into consideration, the use of all kinds of antibiotics in HIV-positive patients with $CD4^+$ counts < 100/μL was not significantly different in those with $CD4^+$ counts > 100/μL (18/37; 48.6% *vs* 29/85; 34.1%, $P = 0.13$) (Table 3). Those antibiotics mainly included Sulfonamides, penicillins and quinolones. None of the patients took NSAIDs, aspirin or steroid before endoscopic examination.

The HIV-positive patients in this study were usually concomitant with HCV and/or HBV infection which was more significantly frequent than in HIV-negative patients (102/122; 83.6% *vs* 2/29; 6.9%, $P < 0.01$). Nine patients with esophagogastric varices (7.4%) and 3 patients with portal hypertensive gastropathy (2.5%) in HIV-positive group were also found by endoscopic examination.

DISCUSSION

In the present study, we found that the majority of gastrointestinal symptoms of HIV-positive patients at our hospital were similar to that of HIV-negative group. In comparison with HIV-negative group, the symptoms of diarrhea, odynophagia, and dysphagia were significantly more in HIV-positive patients ($P < 0.05$). Several previous studies[16,18-19] revealed that more than 71% of AIDS patients who present with dysphagia and odynophagia have endoscopic evidence of esophageal candidiasis. Our result showed a high infection rate of Candida esophagitis in HIV-positive patients (19/122; 15.6%), which may be a possible explanation. Studies showed that the incidence of Cryptosporidium infection has been estimated to be 16%-33% in the AIDS patients in north America with chronic diarrhea[20,21]. In developing countries, the infection of Cryptosporidium was 55% among AIDS patients[22]. The etiological factor of diarrhea in HIV-positive patients in our study was not evaluated.

The prevalence of *H pylori* infection in HIV-positive patients at our hospital was significantly lower than that in HIV-negative control group. Our results were in agreement with some previous reports[8-11]. The reason of lower prevalence of *H pylori* infection may be lack of $CD4^+$ cells, use of antibiotics and proton pump inhibitor, decreased acid secretion, or competitive inhibition by other pathogens in HIV-positive patients.

According to previous reports, $CD4^+$ lymphocytes were reported to be involved[23-25] in the pathogenesis of *H pylori*-related gastritis or ulcer. It is well known that $CD4^+$ cells play a role in inducing gastritis and this gastritis might be a mechanism by which *H pylori* colonization is enhanced[26]. Patients with HIV infection and a low $CD4^+$ count would then lose this mechanism by which *H pylori* colonization is sustained, and infection intensity would diminish. In addition, the T-cell response to the organism could serve to induce tissue and epithelial damage. In AIDS patients, the decreased T-cell would induce a decreased incidence of *H pylori* gastritis[27]. In our results, a stratification of cases on the basis of $CD4^+$ count has shown a decrease of *H pylori* infection with the progression of HIV-related disease, and histological examination revealed less chronic active gastritis in HIV-positive patients than in HIV-negative control group (19.7% *vs* 31%). *H pylori* infection was closely related to chronic active gastritis in HIV-negative group ($P < 0.05$), but not in HIV-positive patients, indicating that other pathogens might exist , such as CMV and Cryptosporidium infection.

An impairment of *H pylori* colonization environment

might result from a progressive atrophic involution of the gastric mucosa with secondary decreased acid secretion in HIV-positive patients, which represents an altered intragastric environment[28,29]. In our results, histologic chronic atrophy gastritis in HIV-positive patients was significantly higher than in HIV-negative group (30/122; 24.6% *vs* 2/29; 6.9%, $P < 0.05$), which might be result of gastric secretory failure in HIV infection patients. The impaired acid secretion may allow subsequent gastric bacterial overgrowth and provide a less suitable environment or competitive inhibition for *H pylori* colonization.

An altered intragastric environment might also result from frequent use of antibiotics against apportunistic infections in patients at an advanced stage of HIV infection[30]. In comparison with HIV-negative group, HIV-positive group had a more frequent use of antibiotics. In HIV-positive patients, previous use of antibiotics with $CD4^+$ counts < 100/μL was not significantly different from that of $CD4^+$ counts > 100/μL ($P = 0.13$), but the prevalence of *H pylori* infection showed significant difference. In our patients, the antibiotics most frequently used was trimetoprim-sulfa, usually for treatment or prophylaxis against pneumocystis in HIV-positive patients, and monotherapy of antibiotics has been proven to inhibit rather than eradicate *H pylori*[31]. Therefore, current prophylaxis has been excluded from evaluation of eradicating the microorganism. Previous use of proton pump inhibitors might alter intragastric environment and therefore influence the prevalence of *H pylori* infection. In the present study, the HIV-negative control group took proton pump inhibitors more frequently than HIV-positive group (13.8% *vs* 2.5%, $P < 0.05$), which further proved the lower prevalence of *H pylori* infection in HIV-positive patients.

In this study, all 122 HIV-positive patients with gastrointestinal symptoms, only 4.1% had peptic ulcer, but 20.7% in HIV-negative group. This might explain that the low prevalence of *H pylori* infection result in the lower incidence of ulcers among HIV-positive patients. On the other hand, decreased acid secretion in HIV-positive patients plays a role in the lower incidence of peptic ulcer. According to previous reports, CMV-associated peptic ulcer disease was highly prevalent and CMV was the only organism significantly associated with gastroduodenal ulcers in HIV-positive patients, and *H pylori* was an uncommon cause of peptic ulcer[11,32]. In our study, among the 5 patients with peptic ulcer in HIV-positive group, only one proved to have CMV infection, which was lower according to previous studies[11,32]. The inadequate biopsies in the present study may be a possible explanation. According to literature, histological changes of CMV infection are patchy in distribution, however, and the single biopsy sensitivity for ulcerative lesions has been reported to be as low as 13%. Therefore, at least 8-10 biopsies of suspicious lesions are recommended[33].

Candida esophagitis is one of the most common opportunistic infections in patients with AIDS[34]. Our study showed that the Candida esophagitis was significantly higher in HIV-positive patients with $CD4^+$ count below 200/μL, and the average $CD4^+$ counts with Candida esophagitis was 116.47 ± 133.08/μL. According to previous studies, the CMV infection is also a common opportunistic pathogen in HIV-positive patients with a low $CD4^+$ count and is one of the main causes of gastrointestinal ulcer in AIDS patients[11,33]. The incidence of CMV infection in HIV-positive patients (4.9%) in our study was lower than previous reports[32]. The incorrect location of biopsy may be another possible reason. According to literature review, gastric CMV infections are usually seen in the fundus with contiguous involvement of the esophagus and gastroesophageal junction, and the distal stomach and antrum are less commonly involved[35,36]. In the present study, the biopsy specimens were usually obtained from gastric antrum and lower body, therefore might lower the incidence of CMV infection in our patients.

The HIV-positive patients in the present study, mainly from Henan Province of China, infected through illegal blood plasma collection, and usually coinfected with HCV and/or HBV infection (83.6%). Endoscopic examination also revealed findings such as esophagogastric varices and portal hypertensive gastropathy, which were significantly different from previous reports.

In summary, we have found that a lower prevalence of *H pylori* infection and peptic ulcer in HIV-positive patients with gastrointestinal symptoms than that of HIV-negative patients with similar symptoms. The mechanism of chronic active gastritis in HIV-positive patients may be different from HIV-negative group that was closely related to *H pylori* infection. Various opportunistic infections (especially Candida esophagitis) of upper gastrointestinal tract likely occur in HIV-positive patients with a $CD4^+$ count less than 200/μL.

COMMENTS

Background

Helicobacter pylori has been proven to be the main cause of chronic gastritis and peptic ulcer in the HIV-negative population. The role and prevalence of *H pylori* infection might be different in the HIV infected patients.

Research frontiers

The immune deficiencies caused by HIV give rise to many different gastrointestinal opportunistic infections, and the prevalence of *H pylori* infection in patients infected with HIV is remarkably low.

Innovations and breakthroughs

It is the first report to characterize the prevalence and role of *H pylori* infection in chronic active gastritis and peptic ulcer in HIV-positive patients infected through illegal blood plasma collection in China, who are usually coinfected with HCV and/or HBV. The pathogen of chronic active gastritis in HIV-positive patients may be different from the general population that was closely related to *H pylori* infection.

Applications

This observation might be of potential value in HIV-positive patients with gastrointestinal symptoms.

Peer review

The authors compared the prevalence of *H pylori* infection, peptic ulcer, cytomegalovirus (CMV) infection and Candida esophagitis in human immunodeficiency virus(HIV)-positive and HIV-negative patients. The lower prevalence of *H pylori* infection and peptic ulcer in HIV-positive patients with gastrointestinal symptoms suggests a different mechanism of peptic ulcerogenesis and a different role of *H pylori* infection in chronic active gastritis and peptic ulcer.

REFERENCES

1 **Carrick J**, Lee A, Hazell S, Ralston M, Daskalopoulos G. Campylobacter pylori, duodenal ulcer, and gastric metaplasia: possible role of functional heterotopic tissue in ulcerogenesis. *Gut* 1989; **30**: 790-797
2 **Suzuki H**, Hibi T, Marshall BJ. Helicobacter pylori: present status and future prospects in Japan. *J Gastroenterol* 2007; **42**: 1-15
3 **Li YY**, Hu PJ, Du GG, Hazell SL. The prevalence of Helicobacter pylori infection in the Peoples Republic of China. *Am J Gastroenterol* 1991; **86**: 446-449
4 **Dooley CP**, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, Blaser MJ. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989; **321**: 1562-1566
5 **Peterson WL**. Helicobacter pylori and peptic ulcer disease. *N Engl J Med* 1991; **324**: 1043-1048
6 **Taylor DE**, Hargreaves JA, Ng LK, Sherbaniuk RW, Jewell LD. Isolation and characterization of Campylobacter pyloridis from gastric biopsies. *Am J Clin Pathol* 1987; **87**: 49-54
7 **Blaser MJ**. Helicobacter pylori: its role in disease. *Clin Infect Dis* 1992; **15**: 386-391
8 **Panos GZ**, Xirouchakis E, Tzias V, Charatsis G, Bliziotis IA, Doulgeroglou V, Margetis N, Falagas ME. Helicobacter pylori infection in symptomatic HIV-seropositive and -seronegative patients: a case-control study. *AIDS Res Hum Retroviruses* 2007; **23**: 709-712
9 **Lichterfeld M**, Lorenz C, Nischalke HD, Scheurlen C, Sauerbruch T, Rockstroh JK. Decreased prevalence of Helicobacter pylori infection in HIV patients with AIDS defining diseases. *Z Gastroenterol* 2002; **40**: 11-14
10 **Cacciarelli AG**, Marano BJ Jr, Gualtieri NM, Zuretti AR, Torres RA, Starpoli AA, Robilotti JG Jr. Lower Helicobacter pylori infection and peptic ulcer disease prevalence in patients with AIDS and suppressed CD4 counts. *Am J Gastroenterol* 1996; **91**: 1783-1784
11 **Chiu HM**, Wu MS, Hung CC, Shun CT, Lin JT. Low prevalence of Helicobacter pylori but high prevalence of cytomegalovirus-associated peptic ulcer disease in AIDS patients: Comparative study of symptomatic subjects evaluated by endoscopy and CD4 counts. *J Gastroenterol Hepatol* 2004; **19**: 423-428
12 **Sud A**, Ray P, Bhasin DK, Wanchu A, Bambery P, Singh S. Helicobacter pylori in Indian HIV infected patients. *Trop Gastroenterol* 2002; **23**: 79-81
13 **Olmos M**, Araya V, Pskorz E, Quesada EC, Concetti H, Perez H, Cahn P. Coinfection: Helicobacter pylori/human immunodeficiency virus. *Dig Dis Sci* 2004; **49**: 1836-1839
14 **Francis ND**, Logan RP, Walker MM, Polson RJ, Boylston AW, Pinching AJ, Harris JR, Baron JH. Campylobacter pylori in the upper gastrointestinal tract of patients with HIV-1 infection. *J Clin Pathol* 1990; **43**: 60-62
15 **Francis ND**, Boylston AW, Roberts AH, Parkin JM, Pinching AJ. Cytomegalovirus infection in gastrointestinal tracts of patients infected with HIV-1 or AIDS. *J Clin Pathol* 1989; **42**: 1055-1064
16 **Dieterich DT**, Wilcox CM. Diagnosis and treatment of esophageal diseases associated with HIV infection. Practice Parameters Committee of the American College of Gastroenterology. *Am J Gastroenterol* 1996; **91**: 2265-2269
17 **Price AB**. The Sydney System: histological division. *J Gastroenterol Hepatol* 1991; **6**: 209-222
18 **Bonacini M**, Young T, Laine L. The causes of esophageal symptoms in human immunodeficiency virus infection. A prospective study of 110 patients. *Arch Intern Med* 1991; **151**: 1567-1572
19 **Bonacini M**, Laine L, Gal AA, Lee MH, Martin SE, Strigle S. Prospective evaluation of blind brushing of the esophagus for Candida esophagitis in patients with human immunodeficiency virus infection. *Am J Gastroenterol* 1990; **85**: 385-389
20 **Rossi P**, Rivasi F, Codeluppi M, Catania A, Tamburrini A, Righi E, Pozio E. Gastric involvement in AIDS associated cryptosporidiosis. *Gut* 1998; **43**: 476-477
21 **Lumadue JA**, Manabe YC, Moore RD, Belitsos PC, Sears CL, Clark DP. A clinicopathologic analysis of AIDS-related cryptosporidiosis. *AIDS* 1998; **12**: 2459-2466
22 **Wyatt SH**, Fishman EK. The acute abdomen in individuals with AIDS. *Radiol Clin North Am* 1994; **32**: 1023-1043
23 **Seifarth C**, Funk A, Reich K, Dahne I, Classen M, Deusch K. Selective increase of CD4+ and CD25+ T cells but not of gamma delta T cells in H. pylori associated gastritis. *Adv Exp Med Biol* 1995; **371B**: 931-934
24 **Bamford KB**, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, Brooks EG, Graham DY, Reyes VE, Ernst PB. Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype. *Gastroenterology* 1998; **114**: 482-492
25 **Edwards PD**, Carrick J, Turner J, Lee A, Mitchell H, Cooper DA. Helicobacter pylori-associated gastritis is rare in AIDS: antibiotic effect or a consequence of immunodeficiency? *Am J Gastroenterol* 1991; **86**: 1761-1764
26 **Bontems P**, Robert F, Van Gossum A, Cadranel S, Mascart F. Helicobacter pylori modulation of gastric and duodenal mucosal T cell cytokine secretions in children compared with adults. *Helicobacter* 2003; **8**: 216-226
27 **Fabris P**, Pilotto A, Bozzola L, Tositti G, Soffiati G, Manfrin V, de Lalla F. Serum pepsinogen and gastrin levels in HIV-positive patients: relationship with CD4+ cell count and Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002; **16**: 807-811
28 **Lake-Bakaar G**, Quadros E, Beidas S, Elsakr M, Tom W, Wilson DE, Dincsoy HP, Cohen P, Straus EW. Gastric secretory failure in patients with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1988; **109**: 502-504
29 **Biasco G**, Miglioli M, Barbara L, Corinaldesi R, di Febo G. Omeprazole, Helicobacter pylori, gastritis, and duodenal ulcer. *Lancet* 1989; **2**: 1403
30 **Pavicic MJ**, Namavar F, Verboom T, van Winkelhoff AJ, De Graaff J. In vitro susceptibility of Helicobacter pylori to several antimicrobial combinations. *Antimicrob Agents Chemother* 1993; **37**: 1184-1186
31 **Peterson WL**, Graham DY, Marshall B, Blaser MJ, Genta RM, Klein PD, Stratton CW, Drnec J, Prokocimer P, Siepman N. Clarithromycin as monotherapy for eradication of Helicobacter pylori: a randomized, double-blind trial. *Am J Gastroenterol* 1993; **88**: 1860-1864
32 **Varsky CG**, Correa MC, Sarmiento N, Bonfanti M, Peluffo G, Dutack A, Maciel O, Capece P, Valentinuzzi G, Weinstock D. Prevalence and etiology of gastroduodenal ulcer in HIV-positive patients: a comparative study of 497 symptomatic subjects evaluated by endoscopy. *Am J Gastroenterol* 1998; **93**: 935-940
33 **Goodgame RW**, Genta RM, Estrada R, Demmler G, Buffone G. Frequency of positive tests for cytomegalovirus in AIDS patients: endoscopic lesions compared with normal mucosa. *Am J Gastroenterol* 1993; **88**: 338-343
34 **Quinn TC**. Clinical approach to intestinal infections in homosexual men. *Med Clin North Am* 1986; **70**: 611-634
35 **Stoane JM**, Haller JO, Orentlicher RJ. The gastrointestinal manifestations of pediatric AIDS. *Radiol Clin North Am* 1996; **34**: 779-790
36 **Wyatt SH**, Fishman EK. The acute abdomen in individuals with AIDS. *Radiol Clin North Am* 1994; **32**: 1023-1043

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RAPID COMMUNICATION

Effects of *H pylori* infection on gap-junctional intercellular communication and proliferation of gastric epithelial cells *in vitro*

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Abstract

AIM: To explore the effects of *H pylori* infection on gap-junctional intercellular communication (GJIC) and proliferation of gastric epithelial cells *in vitro*.

METHODS: A human gastric epithelial cell line (SGC-7901) cultured on coverslips was exposed overnight to intact *H pylori* ($CagA^+$ or $CagA^-$ strains) and sonicated extracts, respectively. GJIC between the cells was detected by fluorescence redistribution after photobleaching (FRAP) technique. Proliferation of SGC cells was determined by methylthiazolyl tetrazolium (MTT) assay.

RESULTS: When compared with control in which cells were cultured with simple medium alone, both $CagA^+$ and $CagA^-$ *H pylori* isolates could inhibit GJIC ($CagA^+$: $F = 57.98$, $P < 0.01$; $CagA^-$: $F = 29.59$, $P < 0.01$) and proliferation ($CagA^+$: $F = 42.65$, $P < 0.01$; $CagA^-$: $F = 58.14$, $P < 0.01$) of SGC-7901 cells. Compared with $CagA^-$ strains, $CagA^+$ *H pylori* more significantly down-regulated GJIC of gastric cells (intact *H pylori*: $t = 13.86$, $P < 0.01$; sonicated extracts: $t = 11.87$, $P < 0.01$) and inhibited proliferation gastric cells to a lesser extent *in vitro* (intact *H pylori*: $t = 3.06$, $P < 0.05$; sonicated extracts: $t = 3.94$, $P < 0.01$).

CONCLUSION: Compared with $CagA^-$ *H pylori* strains, $CagA^+$ strains down-regulate GJIC of gastric epithelial cells more significantly and inhibit proliferation of gastric cells to a lesser extent *in vitro*. *H pylori*, especially $CagA^+$ strains, may play an important role in gastric carcinogenesis.

Key words: *H pylori*; Gap-junctional intercellular communication; Gastric epithelial cell; CagA; Fluorescence redistribution after photobleaching; Methylthiazolyl tetrazolium assay

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INTRODUCTION

Epidemiological and animal studies have demonstrated a strong causal relationship between gastric cancer and chronic infection with *H pylori,* especially cytotoxin-associated gene A (*cagA*)-positive strains[1,2]. The cagA gene product CagA is directly delivered into gastric epithelial cells *via* type Ⅳ secretion system. Following membrane localization and subsequent tyrosine phosphorylation, CagA interacts with a variety of host cell proteins that are involved in the regulation of cell growth and motility[3]. However, the exact mechanism responsible for the development of gastric cancer in *H pylori*-infected patients still remains unclear.

Gap-junctional intercellular communication (GJIC) is an important mechanism controlling cellular homeostasis, proliferation and differentiation. Inhibition of GJIC between adjacent cells has been postulated to be one of the important events occurring during the promotional stage of cancer[4]. The vast majority of neoplastic cells reduce GJIC compared to their nonneoplastic counterparts[5]. A number of tumor promoters, such as 12-O-tetradecanoylphorbol-13-acetate (TPA), have been known as potent inhibitors of GJIC[6].

So far, changes of GJIC in *H pylori*-associated gastric carcinoma have not been extensively exploited. In the present study, we attempted to explore the molecular mechanisms of *H pylori* infection in gastric carcinogenesis by studying its effects on GJIC of gastric epithelial cells *in vitro*.

MATERIALS AND METHODS

H pylori strains

H pylori strains 97002 and 97004 were identified by and stored in Department of Medical Microbiology and

Table 1 Effects of intact *H pylori* and sonicated extracts on GJIC of SGC-7901 cells (*n*, mean ± SE)

Group	CagA$^+$ strain[b]	CagA$^-$ strain[b]
Intact *H pylori*[d]	26.05 ± 3.39 (40)[a]	36.95 ± 3.78 (44)[a]
Sonicated extracts[d]	15.92 ± 2.53 (40)[a]	22.69 ± 2.60 (41)[a]
Negetive control	66.39 ± 9.95 (24)	
Positive control (TPA)	8.47 ± 0.95 (22)	

[b]$P < 0.01$ one-way ANOVA *vs* negative control, [a]$P < 0.05$ ANOVA/Dunnett *vs* negative control, [d]$P < 0.01$ *vs* *t*-test of CagA$^+$ and CagA$^-$ *H pylori* strains.

Table 2 Effect of intact *H pylori* and sonicated extracts on proliferation of SGC-7901 cells (*n*, mean ± SE)

Group	CagA$^+$ strain[b]	CagA$^-$ strain[b]
Intact *H pylori*[d]	0.755 ± 0.048 (6)[a]	0.680 ± 0.036 (6)[a]
Sonicated extracts[d]	0.938 ± 0.037 (6)	0.830 ± 0.056 (6)[a]
Negetive control	0.955 ± 0.038 (6)	
Positive control (TPA)	0.986 ± 0.045 (6)	

[b]$P < 0.01$ one-way ANOVA *vs* negative control, [a]$P < 0.05$ ANOVA/Dunnett *vs* negative control, [d]$P < 0.01$ *vs* *t*-test of CagA$^+$ and CagA$^-$ *H pylori* strains.

Parasitology, Zhejiang University School of Medicine. The genotypes of vacuolating cytotoxin gene A (*vacA*) of the strains 97002 and 97004 were s1a/m1 and m2, respectively. The results of Western blot and cell vacuolation test demonstrated that the strain 97002 was CagA$^+$/VacA$^+$ and 97004 CagA$^-$/VacA$^-$.

H pylori culture

H pylori strains were cultured on ECY blood-free medium[7] at 37℃ for 5 d, under 100% humidity and microaerophilic conditions (50 mL/L O_2, 100 mL/L CO_2, and 850 mL/L N_2). The bacteria were harvested from the agar plates, washed twice with 0.01 mol/L PBS and stored at -20℃.

Preparation of intact H pylori and sonicated extract samples

The frozen bacteria were dissolved in RPMI1640 culture medium and adjusted to 1×10^{10} CFU/L in intact bacterial samples and 1×10^{12} CFU/L in sonicated extract samples, respectively. The preparation of sonicated extract samples additionally included *H pylori* pulverization with ultrasound, centrifugation at 10000 r/min for 20 min with the supernatant collected.

Cell culture

Human gastric epithelial cell line SGC-7901 was obtained from Department of Medical Microbiology and Parasitology, Zhejiang University School of Medicine and cultured in RPMI1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Sijiqing, China), 1×10^5 IU/L penicillin and 100 mg/L streptomycin. The cells were incubated at 37℃ in a humidified atmosphere containing 950 mL/L air and 50 mL/L CO_2. The cells were grown on 22 mm × 22 mm coverslips in tissue culture dishes (35 mm in diameter) and the culture medium was changed every other day. To determine cell proliferation, SGC-7901 cells were plated into 96-well microplates (0.5×10^5 cells/well) and cultured for 12 h.

Cell treatment with H pylori extracts

Twenty-four hours prior to GJIC measurement, cells of the test groups were treated overnight with intact *H pylori* or sonicated extracts. Negative and positive controls were treated with RPMI1640 with 2% NBS and 5 μg/L TPA was added to the positive control during the last 1 h.

Measurement of GJIC by FRAP technique

GJIC between SGC-7901 cells was measured by fluorescence redistribution after photobleaching (FRAP) technique first described in 1986[8]. 6-carboxyfluorescein diacetate (6-CFDA) was used as the dye that could be retained inside the cells due to its hydrolysis by cytoplamic esterases into 6-carboxyfluorescein (6-CF). 6-CF could permeate gap junction channels due to its low molecular weight. FRAP was achieved under a confocal laser scanning microscope (Leica TCS-SP, Germany) and the detailed protocol was performed as previously described[9].

Determination of cell proliferation by MTT assay

When SGC-7901 cells confluenced by 70% in the 96-well microplates, cell proliferation was assessed by methylthiazolyl tetrazolium (MTT) assay as previously described[10]. The absorbance value per well at 570 nm was read on an automatic multiwell spectrophotometer (Bio-Rad, USA).

Statistical analysis

All data were presented as mean ± SE. Statistical analysis was carried out by ANOVA followed by Dunnett's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

H pylori down-regulated GJIC of SGC-7901 cells

The GJIC of SGC-7901 cells was measured by FRAP after treated with intact *H pylori* or sonicated extracts for 24 h and presented as fluorescence transfer rate (K, 10^{-3}/s) (Table 1). In the present study, both CagA$^+$ and CagA$^-$ *H pylori* isolates including intact *H pylori* and sonicated extracts down-regulated GJIC of SGC-7901 cells (CagA$^+$: $F = 57.98$, $P < 0.01$; CagA$^-$: $F = 29.59$, $P < 0.01$). Compared with CagA$^-$ strains, CagA$^+$ *H pylori* more significantly down-regulated GJIC of gastric cells (intact *H pylori*: $t = 13.86$, $P < 0.01$; sonicated extracts: $t = 11.87$, $P < 0.01$). In addition, our study demonstrated that TPA (5 μg/L for 1 h) had a significant inhibitory effect on GJIC of gastric cells.

Effect of H pylori on cell proliferation

The effects of intact *H pylori* and sonicated extracts on the proliferation of SGC-7901 cells were evaluated by MTT assay ($A_{570\ nm}$) (Table 2). The results suggest that both CagA$^+$ and CagA$^-$ *H pylori* isolates inhibited proliferation of SGC-7901 cells (CagA$^+$: $F = 42.65$, $P < 0.01$; CagA$^-$: $F = 58.14$, $P < 0.01$). However, CagA$^+$ *H pylori* strain inhibited proliferation of gastric cells to a lesser extent when compared with CagA$^-$ strain (intact *H pylori*: $t = 3.06$, $P < 0.05$; sonicated extracts: $t = 3.94$, $P < 0.01$).

DISCUSSION

Among various forms of intercellular communication systems in multicellular organisms, GJIC is the only form by which cells exchange signals directly from the inside of one cell to the neighboring cells. GJIC plays a crucial role in maintaining homeostasis by keeping growth control signals at equilibrium among GJIC-connected cells[11,12]. Most tumor cells have a reduced ability to communicate among themselves and/or with surrounding normal cells, confirming the importance of functional GJIC in growth control[13-15]. GJIC is mediated by gap junction channels composed of tetramembrane spanning proteins, known as connexins. At least 13 subtypes of connexin have been identified and four or five subtypes are detectable in the gastrointestinal tract[16].

It has been reported that connexin 32 in normal gastric mucosa is reduced significantly or absent in atrophic gastric mucosa and metaplastic epithelial cells, and no malignant cells from patients with gastric carcinoma contain detectable connexin 32[17,18]. These results suggest that loss of cell-cell communication through the gap junction may act as an early indicator of gastric carcinoma.

In this study, the effects of *H pylori* infection on GJIC of gastric epithelial cells were detected *in vitro*, suppressing interferences of various cytokines and immune factors *in vivo*, suggesting that both $CagA^+$ and $CagA^-$ *H pylori* isolates inhibit GJIC of SGC-7901 cells and the down-regulating effect of $CagA^+$ *H pylori* is more significant than that of $CagA^-$ strains. These findings emphasize the close relationship between *H pylori* especially $CagA^+$ strains and gastric carcinoma.

Increased cellular proliferation rates are characteristic in malignant tissue. Because of unstability of the genome of proliferating cells, hyperproliferation increases the possibility of DNA damage and aneupoidy. Dysplasia may evolve into carcinoma if damaged DNA cannot be repaired on time or fails in promoting the apoptosis system[19]. *H pylori* infection of the gastric mucosa is closely associated with changes in gastric epithelial cell proliferation. *In vivo* data show that gastric epithelial hyperproliferation is common in *H pylori*-infected persons and the degree of proliferation is directly associated with the severity of mucosal neutrophilic infiltration[20-22]. However, it was reported that an overall increase in gastric epithelial cell proliferation is not associated with *H pylori* gastritis[23]. It is not very clear whether the increased proliferation seen *in vivo* is a direct effect of *H pylori*, or a reflex increase in proliferation in response to increased cell damage, indirectly caused by *H pylori*. A recent report by Cabral *et al*[24] suggested that the increased cell proliferation rate in patients with *H pylori* infection might be related to the *H pylori*-induced inflammation rather than to a direct action of the pathogen.

Several *in vitro* studies reported that *H pylori* can inhibit cell proliferation[25,26] , which is consistent with the results of this study. The possible reason for the contradiction between the findings *in vivo* and *in vitro* is that *in vivo* studies are representative of the effect of persistent *H pylori* infection whereas *in vitro* experimental studies are representative of an acute *H pylori*-mediated effect. Also, the increased cell proliferation in patients with *H pylori* infection might be due to the increased production of gastrin *in vivo*[25]. Moreover, *in vivo* increased epithelial cell injury is associated with a reflex increase in proliferation of uninjured cells, which would not be seen *in vitro* as each cultured gastric cell is in contact with bacteria[27]. Cell proliferation is an essential process for the integrity of gastric mucosa. Deceasing cell turnover may increase the chances of ulcer formation and delay ulcer healing. Therefore, our findings seem to be relevant to the pathogenesis of *H pylori*-associated peptic ulcer diseases.

$CagA^+$ *H pylori* is frequently isolated from patients with gastric cancer in Western countries and may be more virulent in its pathogenesis[28,29]. *In vivo* studies reported that infection with $CagA^+$ *H pylori* strains is linked with higher acute inflammatory scores than $CagA^-$ strains[30,31], suggesting that these strains preferentially induce epithelial cell proliferation by stimulating inflammatory mediators. Our results show that $CagA^+$ strains could inhibit proliferation of gastric epithelial cells to a lesser extent than $CagA^-$ ones. Thus gastric cells injured by exposure to $CagA^+$ *H pylori* strains may be more likely to progress through the cell cycle, which possibly results in the risk of replication of cells with DNA damage[27].

In conclusion, *H pylori* can directly inhibit GJIC and proliferation of gastric epithelial cells *in vitro*. Compared with $CagA^-$ *H pylori* strains, $CagA^+$ strains more significantly down-regulate GJIC and inhibit proliferation to a of gastric epithelial cells lesser extent. Accelerated proliferation increases the risk of DNA damage and gene mutation. Inhibited GJIC makes cancer-initiated cells escape from the control of neighboring cells. *H pylori*, especially $CagA^+$ strains, may play an important role in gastric carcinogenesis.

COMMENTS

Background

It has been widely accepted that there is a strong association between *H pylori* infection and gastric cancer, but the exact molecular mechanism of the pathogen in gastric carcinogenesis has not clarified yet. Nearly 40 years ago, loss of functional gap junctions was described in cancer cells and led to the hypothesis that such a type of intercellular communication is involved in the carcinogenesis process. Since then, a lot of data have been accumulated confirming that gap junctions are frequently decreased or absent in cancer cells. Gap junction deficiency has been defined in the literature either as the lack of gap-junction plaques or as the lack of gap-junctional intercellular communication (GJIC). It has been reported that connexin 32 in normal gastric mucosa as a mediator of GJIC is reduced significantly or absent in atrophic gastric mucosa and metaplastic epithelial cells. However, these reports have not revealed the relationship between changed GJIC and *H pylori* infection of the gastric mucosa.

Research frontiers

There has been a considerable interest over recent years in factors that predispose individuals to develop gastric carcinoma. Complex interactions between several *H pylori*, host genetics and environmental factors determine this predisposition. Understanding the molecular mechanism of the interaction between *H pylori* and gastric epithelial cells will provide us with a new strategy for effective prevention of the development of gastric cancer induced by *H pylori* infection.

Innovations and breakthroughs

In this article, the molecular mechanism of *H pylori* infection in gastric carcinogenesis was explored by studying its effects on GJIC of gastric epithelial cells *in vitro*. The results suggest that *H pylori* could inhibit GJIC of cultured gastric epithelial cells and the down-regulation effect on GJIC of $CagA^+$ strains was more significant than $CagA^-$ ones.

Applications

This article emphasizes the close relationship between *H pylori* especially $CagA^+$ strains and gastric carcinoma. It provides a new direction to illuminate the molecular mechanism of *H pylori* in gastric carcinogenesis. It also implies that compounds able to restore GJIC in junctional deficient cells or prevent its disruption in junctional proficient cells may be used in making new strategies for the prevention and/or treatment of human gastric malignancies.

Terminology

Gap junctions: membrane structures made of intercellular channels which permit the diffusion of small hydrophilic molecules from cytoplasm to cytoplasm.

Peer review

The paper seems innovative. Altered expressions of connexins have been observed in various pathological processes of the digestive tract, including gastric cancer. To our knowledge, it is the first study to explore the molecular mechanism of *H pylori* infection in gastric carcinogenesis by studying its effects on GJIC of gastric epithelial cells *in vitro*.

REFERENCES

1 **Kelley JR**, Duggan JM. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol* 2003; **56**: 1-9
2 **Zheng Q**, Chen XY, Shi Y, Xiao SD. Development of gastric adenocarcinoma in Mongolian gerbils after long-term infection with Helicobacter pylori. *J Gastroenterol Hepatol* 2004; **19**: 1192-1198
3 **Hatakeyama M**. The role of Helicobacter pylori CagA in gastric carcinogenesis. *Int J Hematol* 2006; **84**: 301-308
4 **Kang KS**, Yun JW, Yoon B, Lim YK, Lee YS. Preventive effect of germanium dioxide on the inhibition of gap junctional intercellular communication by TPA. *Cancer Lett* 2001; **166**: 147-153
5 **Trosko JE**, Ruch RJ. Cell-cell communication in carcinogenesis. *Front Biosci* 1998; **3**: d208-d236
6 **Ruch RJ**, Trosko JE, Madhukar BV. Inhibition of connexin43 gap junctional intercellular communication by TPA requires ERK activation. *J Cell Biochem* 2001; **83**: 163-169
7 **Fang PC**, Zhu YL, Yin X, Wu QD, Lan MG, Wu PJ. Study on ECY blood-free medium for the isolation of Helicobacter pylori. *Zhonghua Yixue Jianyan Zazhi* 1993; **16**: 131-133
8 **Wade MH**, Trosko JE, Schindler M. A fluorescence photobleaching assay of gap junction-mediated communication between human cells. *Science* 1986; **232**: 525-528
9 **Mao GG**, Fu YT, Ye SJ. Determination of gap junctional intercellular communication in cultured cells. *Zhonghua Laodong Weisheng Zhiyebing Zazhi* 2000; **18**: 376-377
10 **Mosmann T**. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**: 55-63
11 **Yamasaki H**. Role of disrupted gap junctional intercellular communication in detection and characterization of carcinogens. *Mutat Res* 1996; **365**: 91-105
12 **Yamasaki H**, Krutovskikh V, Mesnil M, Tanaka T, Zaidan-Dagli ML, Omori Y. Role of connexin (gap junction) genes in cell growth control and carcinogenesis. *C R Acad Sci III* 1999; **322**: 151-159
13 **Yamasaki H**, Omori Y, Zaidan-Dagli ML, Mironov N, Mesnil M, Krutovskikh V. Genetic and epigenetic changes of intercellular communication genes during multistage carcinogenesis. *Cancer Detect Prev* 1999; **23**: 273-279
14 **Ruch RJ**, Porter S, Koffler LD, Dwyer-Nield LD, Malkinson AM. Defective gap junctional intercellular communication in lung cancer: loss of an important mediator of tissue homeostasis and phenotypic regulation. *Exp Lung Res* 2001; **27**: 231-243
15 **Trosko JE**, Chang CC. Role of stem cells and gap junctional intercellular communication in human carcinogenesis. *Radiat Res* 2001; **155**: 175-180
16 **Nishitani A**, Hirota S, Nishida T, Isozaki K, Hashimoto K, Nakagomi N, Matsuda H. Differential expression of connexin 43 in gastrointestinal stromal tumours of gastric and small intestinal origin. *J Pathol* 2005; **206**: 377-382
17 **Uchida Y**, Matsuda K, Sasahara K, Kawabata H, Nishioka M. Immunohistochemistry of gap junctions in normal and diseased gastric mucosa of humans. *Gastroenterology* 1995; **109**: 1492-1496
18 **Nagahara A**, Watanabe S, Miwa H, Endo K, Hirose M, Sato N. Reduction of gap junction protein connexin 32 in rat atrophic gastric mucosa as an early event in carcinogenesis. *J Gastroenterol* 1996; **31**: 491-497
19 **Gao H**, Wang JY, Shen XZ, Liu JJ. Effect of Helicobacter pylori infection on gastric epithelial cell proliferation. *World J Gastroenterol* 2000; **6**: 442-444
20 **Fraser AG**, Sim R, Sankey EA, Dhillon AP, Pounder RE. Effect of eradication of Helicobacter pylori on gastric epithelial cell proliferation. *Aliment Pharmacol Ther* 1994; **8**: 167-173
21 **Bechi P**, Balzi M, Becciolini A, Maugeri A, Raggi CC, Amorosi A, Dei R. Helicobacter pylori and cell proliferation of the gastric mucosa: possible implications for gastric carcinogenesis. *Am J Gastroenterol* 1996; **91**: 271-276
22 **Murakami K**, Fujioka T, Kodama R, Kubota T, Tokieda M, Nasu M. Helicobacter pylori infection accelerates human gastric mucosal cell proliferation. *J Gastroenterol* 1997; **32**: 184-188
23 **Chow KW**, Bank S, Ahn J, Roberts J, Blumstein M, Kranz V. Helicobacter pylori infection does not increase gastric antrum mucosal cell proliferation. *Am J Gastroenterol* 1995; **90**: 64-66
24 **Cabral MM**, Oliveira CA, Mendes CM, Guerra J, Queiroz DM, Rocha GA, Rocha AM, Nogueira AM. Gastric epithelial cell proliferation and cagA status in Helicobacter pylori gastritis at different gastric sites. *Scand J Gastroenterol* 2007; **42**: 545-554
25 **Ricci V**, Ciacci C, Zarrilli R, Sommi P, Tummuru MK, Del Vecchio Blanco C, Bruni CB, Cover TL, Blaser MJ, Romano M. Effect of Helicobacter pylori on gastric epithelial cell migration and proliferation in vitro: role of VacA and CagA. *Infect Immun* 1996; **64**: 2829-2833
26 **Pearce HR**, Kalia N, Bardhan KD, Atherton JC, Brown NJ. Effects of Helicobacter pylori on endothelial cell proliferation and chemotaxis. *Digestion* 2004; **69**: 201-210
27 **Smoot DT**, Wynn Z, Elliott TB, Allen CR, Mekasha G, Naab T, Ashktorab H. Effects of Helicobacter pylori on proliferation of gastric epithelial cells in vitro. *Am J Gastroenterol* 1999; **94**: 1508-1511
28 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115
29 **Hatakeyama M**, Higashi H. Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci* 2005; **96**: 835-843
30 **Peek RM Jr**, Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC, Blaser MJ. Heightened inflammatory response and cytokine expression in vivo to $cagA^+$ Helicobacter pylori strains. *Lab Invest* 1995; **73**: 760-770
31 **Bhat N**, Gaensbauer J, Peek RM, Bloch K, Tham KT, Blaser MJ, Perez-Perez G. Local and systemic immune and inflammatory responses to Helicobacter pylori strains. *Clin Diagn Lab Immunol* 2005; **12**: 1393-1400

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RAPID COMMUNICATION

Regulation of activin receptor-interacting protein 2 expression in mouse hepatoma Hepa1-6 cells and its relationship with collagen type Ⅳ

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Abstract

AIM: To investigate the regulation of activin receptor-interacting protein 2 (ARIP2) expression and its possible relationships with collagen type Ⅳ (collagen Ⅳ) in mouse hepatoma cell line Hepal-6 cells.

METHODS: The ARIP2 mRNA expression kinetics in Hepal-6 cells was detected by RT-PCR, and its regulation factors were analyzed by treatment with signal transduction activators such as phorbol 12-myristate 13-acetate (PMA), forskolin and A23187. After pcDNA3-ARIP2 was transfected into Hepal-6 cells, the effects of ARIP2 overexpression on activin type Ⅱ receptor (ActRⅡ) and collagen Ⅳ expression were evaluated.

RESULTS: The expression levels of ARIP2 mRNA in Hapel-6 cells were elevated in time-dependent manner 12 h after treatment with activin A and endotoxin LPS, but not changed evidently in the early stage of stimulation (2 or 4 h). The ARIP2 mRNA expression was increased after stimulated with signal transduction activators such as PMA and forskolin in Hepal-6 cells, whereas decreased after treatment with A23187 (25.3% ± 5.7% *vs* 48.1% ± 3.6%, $P < 0.01$). ARIP2 overexpression could remarkably suppress the expression of ActRIIA mRNA in dose-dependent manner, but has no effect on ActRⅡB in Hepal-6 cells induced by activin A. Furthermore, we have found that overexpression of ARIP2 could inhibit collagen Ⅳ mRNA and protein expressions induced by activin A in Hapel-6 cells.

CONCLUSION: These findings suggest that ARIP2 expression can be influenced by various factors. ARIP2 may participate in the negative feedback regulation of signal transduction in the late stage by affecting the expression of ActRIIA and play an important role in regulation of development of liver fibrosis induced by activin.

Key words: Activin receptor-interacting protein 2; Hepal-6 cells; Lipopolysaccharide; Phorbol 12-myristate 13-acetate; Forskolin; Collagen

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INTRODUCTION

Activin is a multifunctional growth and differentiation factor of transforming growth factor-beta (TGF-β) superfamily[1,2]. As an important regulator, activin is involved in the acute phase response of inflammatory diseases and tissue repair, and also play an important role in inducing liver fibrosis[3-5]. The actions of activin on target cells are tissue-specific, which associate with the difference of activin receptor signal transduction. It has been found that the tissue-specificity might depend on a new group of intracellular signal proteins, activin receptor-interacting proteins (ARIPs)[6-8]. ARIPs have four forms at least, all of which can specifically interact with activin type Ⅱ receptor (ActRII) and regulate intracellular signal transduction induced by activin[6-10]. It has been demonstrated that not only the expression and distribution but also the biological activities of ARIPs were obviously different in various tissues. ARIP2 can enhance ActRII endocytosis and reduce ActRIIA receptor expression on cell membranes *via* Ral/RalBP1-depending pathway, and has a capability of suppressing activin-induced signal transduction. There was high expression of ARIP2 mRNA in liver tissues tested by Northern blot[7]. Therefore, we reason out that ARIP2 may participate in the functional regulation of hepatocytes treated by activin.

Since ARIP2 has only been recently discovered, the mode of expression regulation and function of it have not been well characterized. In this study, we have explored

regulation of ARIP2 expression and its effects on the expression of collagen type Ⅳ (collagen Ⅳ) which is component of extracellular matrix (ECM), using mouse Hepal-6 cells, which were obtained from mouse hepatoma cell line and had functions of hepatic parenchymal cells[11].

MATERIALS AND METHODS

Materials

Lipopolysaccharide (LPS, from E.coli 0111:B4), A23187, phorbol 12-myristate 13-acetate (PMA) and forskolin were obtained from Sigma. AMV Reverse Transcriptase was purchased from Promega. ExTaq was obtained from Takara Biotechnology Co (Kyoto, Japan). Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO. Trizol reagent was obtained from Invitrogen. Activin A was provided by Dr. Eto T (Ajinomoto Central Research Laboratories, Japan).

Cell culture

Hepa1-6 cells from mouse hepatoma cell line were provided by Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China) and were maintained in DMEM medium supplemented with 10% fetal calf serum (FCS) at 37℃ in a 5% CO_2 humidified incubator.

Plasmid construction

The vector construction has been described previously[8]. The set of primers was designed as follows. The sense primer was 5'-GGAATTCATGAACGGACGGGTGGATTA-3', which introduced an *EcoR* I site, and the anti-sense primer 5'-GCTCGAGTCATTGTCTGCACAATAAACA-3', which introduced an *Xhol* I site. cDNA fragments encoding full-length ARIP2 (1-153 amino acid residues) were amplified by PCR. The amplified cDNA fragments were inserted into plasmid pMD18-T, and were then subcloned into eukaryotic expression vector pcDNA3. The reconstructed plasmid was named as pcDNA3-ARIP2.

Detection of ARIP2 mRNA expression in Hepal-6 cells stimulated by activin A and LPS

Hepal-6 cells were plated into 12-well tissue culture plates at a density of 2×10^5 cells/mL and incubated in 10% FCS-DMEM at 37℃, 5% CO_2 over night. The cells were cultured in 2% FCS-DMEM in the presence or absence of activin A (5 ng/mL) and LPS (2.5 μg/mL), respectively. After 2, 4, 8, 12 and 24 h, the cells were harvested respectively and total RNA was extracted by using the TRIzol reagent according to the manufacturer's protocol (Invitrogen). The mRNA expression of ARIP2 was examined by RT-PCR, and GAPDH was considered as inner control. PCR was performed for 30 cycles. Amplified PCR products were subjected to 1.5% agarose gel electrophoresis, and stained with ethidium bromide for detection. Specific bands were analyzed using ImageMaster VDS (Pharmacia Biotech Company, Sweden). The primer sequences were shown at Table 1.

Assay of the effects of signal transduction kinetins on the expression of ARIP2 mRNA

To further study the regulation elements of ARIP2 expression, the Hepal-6 cells plated into 12-well tissue culture plates were cultured in 2% FCS-DMEM in the presence or absence of activin A (5 ng/mL), A23187 (200 nmol/L), PMA (20 nmol/L), forskolin (50 μmol/L) and LPS (2.5 μg/mL), respectively. After 24 h, the cells were harvested respectively and total RNA was extracted by using the TRIzol reagent. The expression of ARIP2 mRNA was examined by RT-PCR.

Overexpression of ARIP2 in Hepa1-6 cells

To determine possible bioactivity of ARIP2, effects of ARIP2 on the mRNA expressions of ActRIIA, ActRIIB and collagen type Ⅳ were analyzed by RT-PCR. The Hepal-6 cells were washed once with serum-free DMEM, and were then transfected with pcDNA3-ARIP2 (0.1, 0.3 μg) and pcDNA3 (0.3 μg) by using Lipofectamine 2000 reagent according to the manufacturer's protocol (Invitrogen), respectively. The transfected cells were incubated in the presence or absence of activin A (5 ng/mL) overnight. The cultured cells were harvested and total RNA was extracted by using the TRIzol reagent. RT-PCR was performed for detecting ActRIIA, ActRIIB and type Ⅳ collagen mRNA expressions. The primer sequences were shown at Table 1.

Flow cytometry for type Ⅳ collagen protein expression

Hepal-6 cells were collected 24 h after transfected with pcDNA3-ARIP2. The expression of type Ⅳ collagen proteins were assessed by flow cytometry (FACSort Vantage; BD, Franklin Lakes, NJ) using anti-mouse type Ⅳ collagen antibodies. The data were collected and analyzed on computer (Cell Quest software; BD Biosciences), to assess the percentage of positive fluorescence cells. A representative experiment of the two performed was shown.

RESULTS

Kinetics of ARIP2 mRNA expression in Hepa1-6 cells stimulated by activin A and LPS

As a regulation protein of activin signal pathway, the expression of ARIP2 mRNA could be increased by stimulation with activin A. In this study, the levels of ARIP2 mRNA expression were time-dependently up-regulated 12 h after treatment with activin A in Hepal-6 cells, but not obviously changed at 2-4 h after being treated with activin A. Endotoxin LPS as inflammatory factor can bind with Toll-like receptor 4 on hepatocytes. We found that ARIP2 mRNA expression in Hepal-6 cells was remarkably promoted by LPS treatment, and the expression levels were time-dependently up-regulated 12 h after treatment with LPS in Hepal-6 cells (Figure 1). These data suggested that the expression of ARIP2 was increased in the late stage of activin A and LPS treatment, and ARIP2 might participate in the negative regulation of the late stage signal transduction in Hepal-6 cells.

The signal transduction kinetins regulated the expression of ARIP2 mRNA

PMA is the activator of protein kinase C (PKC)[12], A23187 is the calcium ion vector[13], forskolin is the kinetin of cAMP-dependent protein kinase A (PKA)[14]

Table 1 Primer sequences used in transcriptase-polymerase chain reaction (PCR)

Target	Primers	Sequences	Products size (bp)	Genbank No.
GAPDH	Sense	5′-GATTGTTGCCATCAACGACC-3′	371	BC083149
	Antisense	5′-GTGCAGGATGCATTGCTGAC-3′		
ARIP2	Sense	5′-GTCAGCCGTATCAAAGAGGATG-3′	371	AY157057
	Antisense	5′-CTTGTGGCAATACTTCTCTGGTG-3′		
ActRIIA	Sense	5′-ATTGGCCAGCATCCATCTCTTG-3′	296	XM_123799
	Antisense	5′-TGCCACCATCATAGACTAGATTC-3′		
ActRIIB	Sense	5′-TGCTGAAGAGCGACCTCAC-3′	544	NM_007397
	Antisense	5′-AGCAGGTCCACATTGGTGAC-3′		
Collagen Ⅳ	Sense	5′-GCCTGCTCAAGGAGAAGACA-3′	380	NM_007734
	Antisense	5′-GATCCATAGGAGTCTCCAGGT-3′		

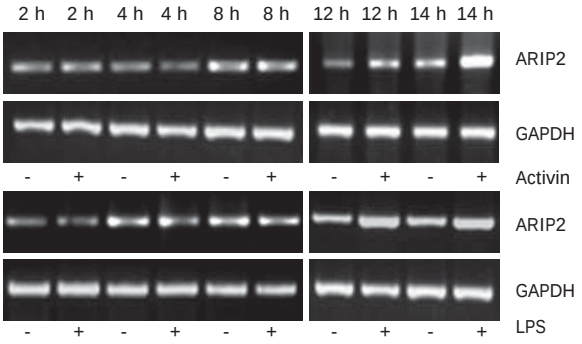

Figure 1 Expressions of ARIP2 mRNA in Hepal-6 cells stimulated by activin A and LPS.

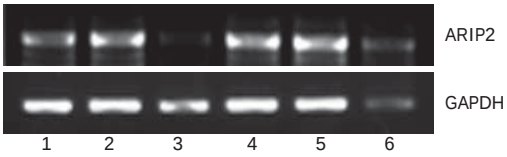

Figure 2 The effects of signal transduction activators on expressions of ARIP2 mRNA. Lane 1: Hepal-6 cells untreated; Lane 2: Treated with activin A (5 ng/mL); Lane 3: A23187 (200 nmol/L); Lane 4: LPS (2.5 μg /mL); Lane 5: PMA (20 nmol/L); Lane 6: forskolin (50 μmol/L).

and endotoxin LPS can bind with Toll-like receptor 4 on the surface of hepatocytes to stimulate cellular activities non-specifically[15]. To further study the regulation factors of ARIP2 expression, we used all of the above signal transduction activators to stimulate Hepal-6 cells and observed the expression of ARIP2 mRNA. The results showed that activin A (ARIP2 mRNA content relative to GAPDH, 66.2% ± 4.9%), LPS (76.5% ± 5.7%), PMA (72.3% ± 5.2%) and forskolin (79.8% ± 6.6%) could promote the expressions of ARIP2 mRNA (untreated control group, 48.1% ± 3.6%), whereas A23187 (25.3% ± 5.7%) could suppress it markedly (Figure 2), 25.3% ± 5.7% *vs* 48.1% ± 3.6%, $P < 0.01$. These data indicated that activators of the PKC, PKA signal transduction pathways and LPS *via* Toll-like receptor 4 could up-regulated the expression of ARIP2 mRNA.

Effects of ARIP2 overexpression on ActRII expression in Hepal-6 cells

To investigate the biological activities of ARIP2 expression in Hepal-6 cells, expression vectors pcDNA3-ARIP2 were

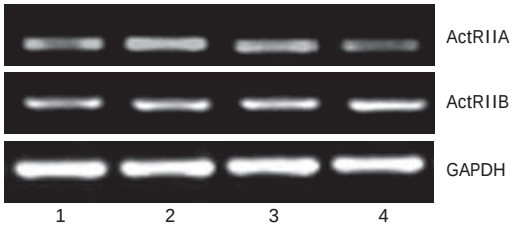

Figure 3 The mRNA expressions of ActRIIA and ActRIIB in ARIP2-overexpressed Hepal-6 cells. Lane 1 and 2: Hepal-6 cells were transfected with empty vector pcDNA3 (0.3 μg); lane 3: pcDNA3-ARIP2 (0.1 μg) + pcDNA3 (0.2 μg); lane 4: pcDNA3-ARIP2 (0.3 μg).

transfected into Hepal-6 cells and the effects of ARIP2 overexpression on ActRIIA and ActRIIB expression were observed in Hepal-6 cells. In this study, we found that ARIP2 overexpression could obviously suppress the expression of ActRIIA mRNA in Hepal-6 cells induced by activin A in dose-dependent manner, but has no effect on ActRIIB (Figure 3). These findings indicated that ARIP2 might down-regulated the expression of ActRIIA to suppress activin signal transduction in hepatocytes.

ARIP2 overexpression suppressed type Ⅳ collagen expression in Hepal-6 cells

The previous studies showed that activin A could induce liver fibrosis and stimulate excess secretion of ECM components, for example, collagen and fibronectin[3,16]. As an inhibitor of activin signal transduction, ARIP2 maybe influence the collagen production in hepatocytes induced by activin A. In this study, activin A could obviously stimulate the expression of type Ⅳ collagen mRNA in Hepal-6 cells. Whereas, after transfecting pcDNA3-ARIP2 into Hepa1-6 cells for 24 h, the ARIP2 overexpression could significantly suppress the expressions of type Ⅳ collagen mRNA induced by activin A in dose-dependent manner (Figure 4). To further determine the type Ⅳ collagen protein expression, the mature type Ⅳ collagen protein levels in Hepa1-6 cells were examined by flow cytometry. The results showed that ARIP2 overexpression could remarkably inhibit the expression levels of type Ⅳ collagen proteins in Hepa1-6 cells induced by activin A (the percent of positive fluorescence cells, 2% *vs* 16%) (Figure 5), which results were the same with that of type Ⅳ collagen mRNA by RT-PCR. These findings suggested that high level expression of ARIP2 might influence the expression of ECM components in hepatocytes and

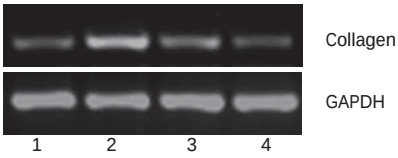

Figure 4 ARIP2-overexpression suppressed the expressions of collagen Ⅳ mRNA in Hepal-6 cells. lane 1 and 2: Hepal-6 cells were transfected with empty vector pcDNA3 (0.3 μg); lane 3: pcDNA3-ARIP2 (0.1 μg) + pcDNA3 (0.2 μg); lane 4: d pcDNA3-ARIP2 (0.3 μg). The cells were incubated in the absence (lane 1) or the presence of activin A (5 ng/mL) (lane 2, 3 and 4) for 24 h.

down-regulate the development of liver fibrosis induced by activin.

DISCUSSION

ARIPs have obvious expression and distribution diversity, which are key factors to the histological specificity of activin action[6-8]. It has been demonstrated that both ARIP1 and ARIP2 are inhibitors of activin signal transduction. However, ARIP1 mainly distributed in nerve tissues, ARIP2 widely existed in tissues. The high expression of ARIP2 mRNA could be detected in liver tissue by Northern blot[7]. In order to investigate the kinetic changes of ARIP2 expression, we used activin A and endotoxin LPS to stimulate Hepal-6 cells, and then examined the expression of ARIP2 mRNA. The results showed that the expression levels of ARIP2 mRNA were not changed obviously after being treated by activin in the early stage, but up-regulated depending on time 12 to 24 h after treatment (Figure 1). Stimulated by LPS, ARIP2 expression is also up-regulated evidently 12 h after treatment. In the present study, we further examined the effects of signal transduction activators PMA, A23187 and forskolin on the expression of ARIP2 in Hepal-6 cells (Figure 2). These data indicated that activin A, LPS, PMA and forskolin could promote the expression of ARIP2 mRNA in Hepal-6 cell, whereas calcium ion vector-A23187 could inhibit the expression of ARIP2 mRNA. These findings suggested that ARIP2 expression could be influenced by various factors and might participate in the regulation of signal transduction in the late stage in Hepal-6 cells.

Activin receptors are the members of serine/threonine kinase receptors[9,10]. Activin binds to receptor type Ⅱ to form a complex primarily. The complex interacts with the receptor type I and makes it phosphorylated, then activates endocellular Smad2/3 protein binding to receptor type I . Finally, it transduces signal into nucleus mediated by Smad4. Therefore, ActRIIs are the crucial receptors of activin signal transduction. In this study, we found that both ActR IIA and ActR IIB could be expressed in heapl-6 cells. To investigate the biological activities of ARIP2 expression in Hepal-6 cells, we transfected Hepal-6 cells with pcDNA3-ARIP2 and observed the effect of ARIP2 overexpression on ActRIIA and ActRIIB expressions in Hepal-6 cells. The results showed that ARIP2 overexpression could obviously suppress the expression of ActRIIA mRNA in Hepal-6 cells induced by activin A, but had no effect on ActRIIB (Figure 3). All the above data indicated that ARIP2 could down-regulated the expression of ActRIIA and participate in the process of negative feedback regulation of activin signal in hepatocytes.

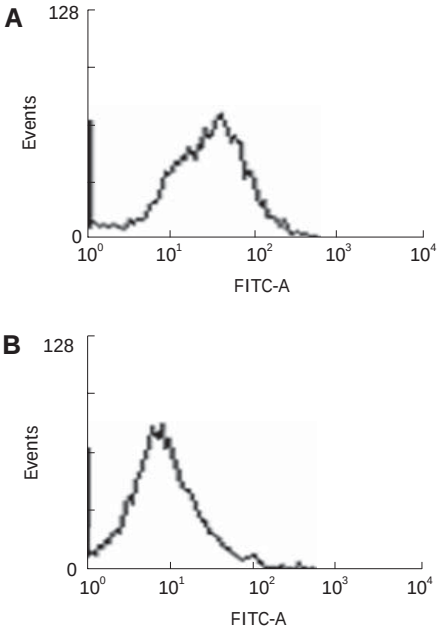

Figure 5 Flow cytometry analysis of collagen Ⅳ protein expressions in Hepal-6 cells. **A**: Hepal-6 cells were transfected with 0.3 μg pcDNA3; **B**: with 0.3 μg pcDNA3-ARIP2. 16% or 2% expressed the percent of positive fluorescence cells in **A** or **B**.

Activin not only play an important role in regulating secretion of hormone, but also serves as autocrine and paracrine factors to regulate the differentiation, proliferation, apoptosis of cells and embryonic development[17-21]. The latest studies have reported that as an important regulator, activin also has effects on inducing liver fibrosis, suppressing hepatocyte growth and so on[21-26]. It has been demonstrated that activin was produced by hepatocyte and hepatic stellate cell (HSC) and could promote HSC activation and stimulate excess production of ECM components, for example, collagen and fibronectin[3,16,25]. It has been reported that activin A could be expressed positively in fibrotic hepatocytes, and it also could take actions by autocrine[3,26]. In this study, we found that activin A could stimulate the expression of type Ⅳ collagen mRNA, whereas, the ARIP2 overexpression could remarkably suppress the mRNA expression of type Ⅳ collagen in Hepal-6 cells induced by activin A (Figure 4) and decrease the protein expression levels of type Ⅳ collagen. As a kind of collagen composed ECM, type Ⅳ collagen could co-deposited in Diss with fibronectin in the early stage of hepatic injury and take part in the formation of liver fibrosis. These findings suggested that ARIP2 overexpression might influence the synthesis of ECM in hepatocyte and negatively regulate the formation and development of liver fibrosis induced by activin A.

In conclusion, ARIP2 can be up-expressed in Hepal-6 cells by inducement with various factors and may participate in the regulation of signal transduction in the late stage. We may release or restraint liver diseases induced

by activin and achieve the goal of treatment if ARIP2 expression can be elevated in hepatocytes to inhibit the effects of activin.

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COMMENTS

Background

Activin A is involved in hepatic fibrosis formation. However, the mechanism of fibrotic process is not well understood. In this study, effects of anti-fibrosis by ARIP2 are investigated in mouse Hepal-6 cells.

Research frontiers

ARIP2 is a regulator of activin signaling pathway, but studies about its regulation in production of component of extracellular matrix (ECM)s are not reported.

Innovations and breakthroughs

Since ARIP2 has only been recently discovered, the mode of expression regulation and function of it have not been well characterized. We designed this experiment to investigate ARIP2 expression and its effects on the expression of collagen type Ⅳ by using Hepal-6 cells.

Applications

No ideal drug is available so far for the therapy of hepatic fibrosis. ARIP2 may be play an important role in regulation of development of liver fibrosis induced by activin.

Terminology

Activin receptor-interacting protein2 (ARIP2) can specifically interact with activin type Ⅱ receptor (ActRⅡ) and down-regulate intracellular signal transduction induced by activin.

Peer review

The topic is of interest for that up to now no antifibrotic therapy is available in patients with hepatic fibrosis. The negative effect of ARIP2 on production of component of extracellular matrix described in this paper shows that ARIP2 might be a potential treatment option.

REFERENCES

1 **Vale W**, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J. Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature* 1986; **321**: 776-779
2 **Kingsley DM**. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes Dev* 1994; **8**: 133-146
3 **Wada W**, Kuwano H, Hasegawa Y, Kojima I. The dependence of transforming growth factor-beta-induced collagen production on autocrine factor activin A in hepatic stellate cells. *Endocrinology* 2004; **145**: 2753-2759
4 **Endo D**, Maku-Uchi M, Kojima I. Activin or follistatin: which is more beneficial to support liver regeneration after massive hepatectomy? *Endocr J* 2006; **53**: 73-78
5 **Werner S**, Alzheimer C. Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine Growth Factor Rev* 2006; **17**: 157-171
6 **Shoji H**, Tsuchida K, Kishi H, Yamakawa N, Matsuzaki T, Liu Z, Nakamura T, Sugino H. Identification and characterization of a PDZ protein that interacts with activin type II receptors. *J Biol Chem* 2000; **275**: 5485-5492
7 **Matsuzaki T**, Hanai S, Kishi H, Liu Z, Bao Y, Kikuchi A, Tsuchida K, Sugino H. Regulation of endocytosis of activin type II receptors by a novel PDZ protein through Ral/Ral-binding protein 1-dependent pathway. *J Biol Chem* 2002; **277**: 19008-19018
8 **Liu ZH**, Tsuchida K, Matsuzaki T, Bao YL, Kurisaki A, Sugino H. Characterization of isoforms of activin receptor-interacting protein 2 that augment activin signaling. *J Endocrinol* 2006; **189**: 409-421
9 **Tsuchida K**, Matsuzaki T, Yamakawa N, Liu Z, Sugino H. Intracellular and extracellular control of activin function by novel regulatory molecules. *Mol Cell Endocrinol* 2001; **180**: 25-31
10 **Tsuchida K**, Nakatani M, Matsuzaki T, Yamakawa N, Liu Z, Bao Y, Arai KY, Murakami T, Takehara Y, Kurisaki A, Sugino H. Novel factors in regulation of activin signaling. *Mol Cell Endocrinol* 2004; **225**: 1-8
11 **Darlington GJ**. Liver cell lines. *Methods Enzymol* 1987; **151**: 19-38
12 **Yokoyama G**, Fujii T, Tayama K, Yamana H, Kuwano M, Shirouzu K. PKCdelta and MAPK mediate G(1) arrest induced by PMA in SKBR-3 breast cancer cells. *Biochem Biophys Res Commun* 2005; **327**: 720-726
13 **Shaposhnikova VV**, Egorova MV, Kudryavtsev AA, Levitman MKh, Korystov YuN. The effect of melittin on proliferation and death of thymocytes. *FEBS Lett* 1997; **410**: 285-288
14 **Hoshi T**, Garber SS, Aldrich RW. Effect of forskolin on voltage-gated K+ channels is independent of adenylate cyclase activation. *Science* 1988; **240**: 1652-1655
15 **Hoshino K**, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999; **162**: 3749-3752
16 **Date M**, Matsuzaki K, Matsushita M, Tahashi Y, Sakitani K, Inoue K. Differential regulation of activin A for hepatocyte growth and fibronectin synthesis in rat liver injury. *J Hepatol* 2000; **32**: 251-260
17 **Thomsen G**, Woolf T, Whitman M, Sokol S, Vaughan J, Vale W, Melton DA. Activins are expressed early in Xenopus embryogenesis and can induce axial mesoderm and anterior structures. *Cell* 1990; **63**: 485-493
18 **Liu ZH**, Shintani Y, Sakamoto Y, Harada K, Zhang CY, Fujinaka Y, Abe M, Goto T, Saito S. Effects of LHRH, FSH and activin A on follistatin secretion from cultured rat anterior pituitary cells. *Endocr J* 1996; **43**: 321-327
19 **Zhang XJ**, Li Y, Tai GX, Xu GY, Zhang PY, Yang Y, Lao FX, Liu ZH. Effects of activin A on the activities of the mouse peritoneal macrophages. *Cell Mol Immunol* 2005; **2**: 63-67
20 **Takamura K**, Tsuchida K, Miyake H, Tashiro S, Sugino H. Activin and activin receptor expression changes in liver regeneration in rat. *J Surg Res* 2005; **126**: 3-11
21 **Chen W**, Woodruff TK, Mayo KE. Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins. *Endocrinology* 2000; **141**: 1263-1272
22 **Kanamaru C**, Yasuda H, Fujita T. Involvement of Smad proteins in TGF-beta and activin A-induced apoptosis and growth inhibition of liver cells. *Hepatol Res* 2002; **23**: 211-219
23 **Yuen MF**, Norris S, Evans LW, Langley PG, Hughes RD. Transforming growth factor-beta 1, activin and follistatin in patients with hepatocellular carcinoma and patients with alcoholic cirrhosis. *Scand J Gastroenterol* 2002; **37**: 233-238
24 **Hughes RD**, Evans LW. Activin A and follistatin in acute liver failure. *Eur J Gastroenterol Hepatol* 2003; **15**: 127-131
25 **De Bleser PJ**, Niki T, Xu G, Rogiers V, Geerts A. Localization and cellular sources of activins in normal and fibrotic rat liver. *Hepatology* 1997; **26**: 905-912
26 **Huang X**, Li DG, Wang ZR, Wei HS, Cheng JL, Zhan YT, Zhou X, Xu QF, Li X, Lu HM. Expression changes of activin A in the development of hepatic fibrosis. *World J Gastroenterol* 2001; **7**: 37-41

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RAPID COMMUNICATION

Effects of large dose of dexamethasone on inflammatory mediators and pancreatic cell apoptosis of rats with severe acute pancreatitis

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Abstract

AIM: To investigate the influence of high dose of dexamethasone on inflammatory mediators and apoptosis of rats with severe acute pancreatitis (SAP).

METHODS: SAP rats were randomly assigned to the model group and treatment group while the normal rats were assigned to the sham operation group. The mortality, ascite volumes, ascites/body weight ratio and pancreas pathological changes of all rats were observed at 3, 6 and 12 h after operation. Their contents of amylase and endotoxin in plasma and contents of tumor necrosis factor (TNF-α), phospholipase A_2 (PLA_2) and IL-6 in serum were also determined. The microarray sections of their pancreatic tissues were prepared, terminal transferase dUTP nick end labeling (TUNEL) staining was performed and apoptotic indexes were calculated.

RESULTS: There was no marked difference between treatment group and model group in survival. The contents of amylase and endotoxin in plasma and contents of TNF-α, PLA_2 and IL-6 in serum, ascite volumes, ascites/body weight ratio and pancreas pathological scores were all lower in treatment group than in model group to different extents at different time points [$P < 0.05$, 58.3 (26.4) ng/L *vs* 77.535 (42.157) ng/L in TNF-α content, 8.00 (2.00) points *vs* 9.00 (2.00) points in pathological score of pancreas respectively; $P < 0.01$, 0.042 (0.018) EU/mL *vs* 0.056 (0.0195) EU/mL in endotoxin content, 7791 (1863) U/L *vs* 9195 (1298) U/L in plasma amylase content, 1.53 (0.79) *vs* 2.38 (1.10) in ascites/body weight ratio, 8.00 (1.00) points *vs* 11.00 (1.50) points in pathological score of pancreas; $P < 0.001$, 3.36 (1.56) ng/L *vs* 5.65 (1.08) ng/L in IL-6 content, 4.50 (2.00) *vs* 7.20 (2.00), 4.20 (1.60) *vs* 6.40 (2.30), 3.40 (2.70) *vs* 7.90 (1.70) in ascite volumes, respectively]. The apoptotic indexes of pancreas head and pancreas tail were all higher in treatment group than in model group at 6 h [$P < 0.01$, 0.00 (2.00)% *vs* 0.00 (0.00)%, 0.20 (1.80) *vs* 0.00 (0.00) in apoptosis indexes, respectively].

CONCLUSION: The mechanism of dexamethasone treatment in acute pancreatitis is related to its inhibition of inflammatory mediator generation and induction of pancreatic acinar cell apoptosis.

Key words: Severe acute pancreatitis; Apoptosis; Inflammatory mediators; Dexamethasone; Tissue microarrays

Zhang XP, Chen L, Hu QF, Tian H, Xu RJ, Wang ZW, Wang KY, Cheng QH, Yan W, Li Y, Li QY, He Q, Wang F. Effects of large dose of dexamethasone on inflammatory mediators and pancreatic cell apoptosis of rats with severe acute pancreatitis. *World J Gastroenterol* 2007; 13(41): 5506-5511

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INTRODUCTION

The pathogenesis of severe acute pancreatitis (SAP) is closely related to the factors such as activation of pancreatin, release of inflammatory mediators, microcirculation

disturbance and apoptosis. The sound therapeutic effects of large dose of dexamethasone on SAP have been demonstrated. In this experiment, the mechanism of large dose of dexamethasone in SAP was discussed and the changes of inflammatory mediator content and pancreatic acinar cell apoptosis after dexamethasone treatment for SAP rats were observed. The tissue microarray has also been applied to the pathohistological examination of pancreatitis to improve the study efficiency.

MATERIALS AND METHODS

Materials

Clean grade healthy male Sprague-Dawley (SD) rats with body weight of 250-300 g were purchased from the Experimental Animal Center of Medical School, Zhejiang University. Sodium taurocholate and pentobarbital were purchased from USA Sigma Company, and dexamethasone injection from Zhejiang Xinchang Pharmaceutical Company, China. The full automatic biochemical analyzer was used to determine the plasma amylase level (U/L). Plasma endotoxin tachypleus amebocyte lysate kit was purchased from Shanghai Yihua Medical Science and Technology Corporation (Institute of Medical Analysis, Shanghai, China), the calculation unit is EU/mL. The TNF-α ELISA kit was purchased from Jingmei Bioengineering Corporation, the calculation unit is pg/mL (ng/L). The serum secretory phospholipase A_2 enzyme assay ELA kit (PLA_2) was purchased from R&D System Institute and the calculation unit is U/mL. The above determinations were all operated according to the instructions of the kits.

Animal grouping and rat SAP model preparation

Ninety clean grade healthy male SD rats were prepared into SAP models by the improved Aho's method and randomly divided into the model group (45 rats) and treatment group (45 rats). Another 45 were assigned into the sham operation group. The above groups were then randomly divided into the 3, 6 and 12 h group with 15 rats in each. The treatment group was injected with dexamethasone via vena caudalis, 0.5 mg/100 g body weight, 15 min after successful preparation of SAP model. In the sham operation group, pancreas and duodenum were turned over before the abdomen was closed. The sham operation group and model group were injected with the saline of the same volume *via* vena caudalis 15 min after the operation[1]. SAP model was established according to the reference[1].

Observation indexes

The rat mortality was determined at 3, 6 and 12 h after operation and the survival rate was calculated at different time points.

After the rats were anesthetized by sodium pentobarbital and killed in batches, the pancreas samples were collected. Fix them according to the related requirements, observe the pathological changes of pancreas after HE staining and compare the pathological scores among groups. The standard of pancreas pathological score was in accordance with reference[2]. The content of amylase, endotoxin in plasma, and TNF-α, IL-6 and PLA_2 in serum of all groups were determined at different time points.

Table 1 Comparison of ascite volumes [M (Q_R)]

Group	3 h	6 h	12 h
Sham operation group	0.50 (0.00)	0.70 (0.50)	0.60 (0.30)
Model group	7.20 (2.00)	6.40 (2.30)	7.90 (1.70)
Dexamethasone treated group	4.50 (2.00)[b]	4.20 (1.60)[b]	3.40 (2.70)[b]

[b]$P < 0.001$, dexamethasone treated group *vs* model group.

Apoptotic indexes

The tissue microarray was applied to prepare the tissue microarray sections of pancreas, which were stained by DNA, and terminal transferase dUTP nick end labeling (TUNEL). The observation of pancreatic cells and calculation of apoptotic indexes were carried out respectively.

Statistical analysis

The statistical analysis was conducted with the SPSS11.5 software. The Kruskal-Wallis test or variance analysis (only applied to PLA_2) was performed for the comparison among the three groups. The Bonfferoni test was also applied to the comparison. There are statistical significances when $P < 0.05$.

RESULTS

Survival

The mortality of model group was 0% (0/15), 0% (0/15) and 13.33% (2/15) at 3, 6 and 12 h, respectively. The sham operation group and dexamethasone treated group survived at all time points while there was no marked difference between the model group and dexamethasone treated group ($P > 0.05$)[1].

Comparison of ascite volumes

The model group and treated group had significantly higher ascite columes than sham operation group ($P < 0.001$), while the treatment group had significantly lower ascite volume than the model group ($P < 0.001$) (Table 1).

Comparison of ascites/body weight ratio

The model group and treatment group had significantly higher ascites/body weight ratio than sham operation group ($P < 0.001$), while the treatment group had significantly lower ratio than the model group at 3 h ($P < 0.01$), and the treatment group had significantly lower ratio than the model group at 6 and 12 h ($P < 0.001$) (Table 2).

Comparison of plasma amylase content

The plasma amylase content in model group and dexamethasone treated group was significantly higher than in the sham operation group at all time points ($P < 0.001$). There was no marked difference between the dexamethasone treated group and model group at 3

Table 2 Comparison of ascites/body weight ratio [M (Q_R)]

Group	3 h	6 h	12 h
Sham operation group	0.20 (0.04)	0.30 (0 .30)	0.22 (0.10)
Model group	2.38 (1.10)	2.58 (0.70)	2.54 (0.71)
Dexamethasone treated group	1.53 (0.79)[b]	1.40 (0.63)[d]	1.36 (0.74)[d]

[b]$P < 0.01$, [d]$P < 0.001$, dexamethasone treated group *vs* model group.

and 6 h ($P > 0.05$). The plasma amylase content in the dexamethasone treated group was significantly less than in the model group at 12 h ($P < 0.01$) (Table 3).

Comparison of plasma endotoxin content

The plasma endotoxin content in the model group and dexamethasone treated group was significantly higher than in the sham operation group at all time points ($P < 0.001$). No marked difference was found between the dexamethasone treated group and model group at 3 h ($P > 0.05$). The content in dexamethasone treated group was significantly less than in the model group at 6 and 12 h ($P < 0.01$) (Table 3).

Comparison of serum TNF-α content

The model group and dexamethasone treated group had significantly higher serum TNF-α content than the sham operation group at all time points ($P < 0.001$). No marked difference was noticed between the dexamethasone treated group and model group at 3 h ($P > 0.05$). The dexamethasone treated group had significantly less serum TNF-α content than the model group at 6 and 12 h ($P < 0.05$)[1] (Table 3).

Comparison of serum IL-6 content

The serum IL-6 contents of model group and treatment group were significantly higher than those of sham operation group ($P < 0.001$); the content of treatment group was significantly lower than that of model group at 3 h ($P < 0.01$); and the contents of treatment group were significantly lower than those of model group at 6 and 12 h ($P < 0.001$) (Table 3).

Comparison of serum PLA_2 content

The model group and dexamethasone treated group significantly higher serum PLA_2 content than the sham operation group at all time points ($P < 0.001$). The content in the dexamethasone treated group was significantly less than in the model group ($P < 0.001$) (Table 4).

Pathological score of pancreatic tissue

HE staining was performed and the pathohistological score standard was referred to the improved Schmidt score. Two chief pathologists used the blind method for scoring[2].

Gross pathological changes of pancreas. (1) Sham operation group: No apparent abnormality of pancreas and peripancreatic epiploon at all time points. (2) Model group: The gross pathological change of pancreas tail was more apparent than that of pancreas head. The severity of overall pathological change increased with time after modeling. At 3 h, a small amount of hemorrhagic ascites was observed by naked eyes with relatively apparent changes of pancreas hyperemia and edema, hemorrhage and necrosis; at 6 and 12 h, hemorrhagic ascites increased more apparently with edema, hemorrhage and necrosis, and more saponified spots could be seen on peripancreatic epiploon and peritoneum. (3) Treatment group: At 3 h, the degree of pancreas hyperemia and edema, hemorrhage and necrosis was milder than that of model group with decrease of ascitic fluid; at 6 and 12 h, the pancreatic hemorrhage and necrosis area and degree were milder than those of model group with apparent decrease of ascitic fluid.

The pancreas pathological changes under light microscope. (1) Sham operation group: Mild interstitial edema occurred in a few cases, and neutrophil infiltration was occasional. No acinar cell, fat necrosis and hemorrhage were observed. (2) Model group: The pathological change severity increased with time after modeling. At 3 h, pancreas interstitial hyperemia, edema, a small amount of inflammatory cell infiltration, focal necrosis and interstitial hemorrhage occurred, among which some were lamellar hemorrhage and necrosis. At 6 h, interstitial edema, hemorrhage, inflammatory cell infiltration, focal and lamellar hemorrhage and necrosis occurred. At 12 h, large area of hemorrhage and necrosis, lobule outline damage and a large amount of inflammatory cell infiltration were found. (3) Treatment group: The pathological change scope and degree of most cases were milder than those of model group at corresponding time points. Only a few had lamellar hemorrhage and necrosis, but the scope of hemorrhage and necrosis decreased and inflammatory cell infiltration apparently alleviated.

Comparison of pathological score of pancreas

Both model group and dexamethasone group had significantly higher pathological score of pancreas than the sham operation group at different time points ($P < 0.01$) while that in dexamethasone group was significantly less than in the model group at 3 and 6 h ($P < 0.05$), and it was also significantly less in the dexamethasone group than in the model group at 12 h ($P < 0.01$) (Table 5).

Comparison of apoptosis indexes

The apoptosis index of pancreas head and tail at 3 and 12 h was not significantly different among all groups ($P > 0.05$). No marked difference was found between the model group and sham operation group at different time points ($P > 0.05$). At 6 h, the apoptosis index of pancreas in the treatment group was significantly higher in the model group and sham operation group ($P < 0.01$) (Table 6, Figure 1A and B).

Correlation analysis

There was a positive correlation between amylase and PLA_2 of model group at 3 h ($P < 0.05$); the TNF-α content of treatment group was positively correlated with PLA_2 at 6 h ($P < 0.05$). There was a positive correlation between pancreas pathological score and TNF-α ($P < 0.05$).

Table 3 Comparison of different indexes level in blood [M (Q_R)]

Index	Sham operation group			Model group			Dexamethasone treated group		
	3 h	6 h	12 h	3 h	6 h	12 h	3 h	6 h	12 h
Amylase	2038	2117	1725	7423	8149	9195	6739	7839	7791[b]
(U/L)	(346)	(324)	(434)	(2275)	(1540)	(1298)	(2310)	(2258)	(1863)
Endotoxin	0.015	0.015	0.016	0.035	0.055	0.056	0.03	0.040[b]	0.042[b]
(EU/mL)	(0.007)	(0.007)	(0.005)	(0.017)	(0.025)	(0.0195)	(0.014)	(0.012)	(0.018)
TNF-α	3.3	4.9	3.7	46.125	77.535	67.301	38.4	58.3[a]	38.7[a]
(ng/L)	(3.6)	(2.6)	(2.3)	(37.954)	(42.157)	(32.1315)	(26.6)	(26.4)	(28.5)
IL-6	1.75	1.75	1.48	4.87	6.65	5.65	3.31[b]	3.17[b]	3.36[b]
(ng/L)	(0.65)	(1.04)	(0.57)	(1.38)	(1.45)	(1.08)	(1.38)	(1.28)	(1.56)

[a]$P < 0.05$, [b]$P < 0.01$, dexamethasone treated group *vs* model group.

Table 4 Comparison of serum PLA_2 content (mean ± SD, U/mL)

Group	3 h	6 h	12 h
Sham operation group	18.70 ± 4.40	16.70 ± 3.83	18.52 ± 11.32
Model group	103.70 ± 20.82	119.85 ± 17.74	121.29 ± 17.00
Dexamethasone treated group	53.96 ± 15.4[b]	67.75 ± 27.95[b]	65.27 ± 26.21[b]

[b]P < 0.001, dexamethasone treated group *vs* model group.

Table 5 Comparison of pathological score of pancreas [M (Q_R)]

Group	3 h	6 h	12 h
Sham operation group	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Model group	8.00 (2.00)	9.00 (2.00)	11.00 (1.50)
Dexamethasone treated group	7.00 (2.00)[a]	8.00 (2.00)[a]	8.00 (1.00)[b]

[a]$P < 0.05$, [b]$P < 0.01$, dexamethasone treated group *vs* model group.

Table 6 Comparison of apoptosis index of the head and tail of pancreas [M (Q_R)]

Group (*t*/h)	Pancreas head			Pancreas tail		
	3 h	6 h	12 h	3 h	6 h	12 h
Sham operationgroup	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Dexamethasone treated group	0.00 (0.00)	0.00 (2.00)[b]	0.00 (0.00)	0.00 (0.00)	0.20 (1.80)[b]	0.00 (0.00)

[b]$P < 0.01$, dexamethasone treated group *vs* model group.

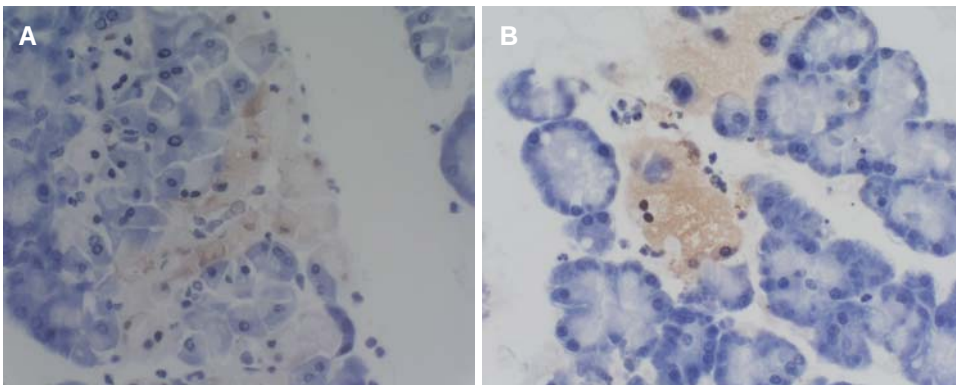

Figure 1 **A**: Dexamethasone treated group-6 h (Apoptosis of pancreatic acinar cell); **B**: Dexamethasone treated group-6 h (Apoptosis of pancreatic acinar cell). (TUNEL × 400).

DISCUSSION

Under normal circumstances, the inflammatory mediators are at low level of dynamic balance to maintain the stability of the internal environment. Excessive inflammatory reaction plays a vital role in SAP pathogenesis[3-6]. In this experiment, the influence of dexamethasone on inflammatory mediators in treatment of SAP rats was studied and its relationship with the apoptosis of pancreatic acinar cells was discussed.

TNF-α can increase the local tissue damage and capillary permeability, eventually aggravating pancreatic necrosis, which is important in AP[7,8]. Norman *et al*[7] found in SAP rats a positive correlation between the TNF-α concentrations in pancreatic tissue and plasma and the level of pancreatic injury and inflammation, which is consistent to the fact that in this experiment, the pancreas pathological score was positively correlated with TNF-α at 12 h in model group ($P < 0.05$). It was found in this experiment that the serum TNF-α contents were lower

in treatment group than in model group at 6 and 12 h ($P < 0.05$), demonstrating that dexamethasone plays a certain role in inhibiting serum TNF-α content. Since PLA_2 plays an important role in SAP onset[8-10], PLA_2 antagonist can significantly improve the pathological injury of pancreas of the animal model with pancreatic injury[11,12]. In this study, the serum PLA_2 content was lower in treatment group than in model group ($P < 0.001$), demonstrating a significant inhibiting effect of dexamethasone on PLA_2 or its generation. IL-6, mainly generated by monocyte/macrophage, T cell, B cell, *etc*, participates in many acute body reactions such as burn, sepsis and major operation. The positive correlation between serum IL-6 level and AP severity has been proved by many studies[13]. And the histological score of pancreas can be significantly improved by lowering IL-6. The serum IL-6 contents were all lower in treatment group than in model group to different extents ($P < 0.01$ or $P < 0.001$), demonstrating that dexamethasone can inhibit serum IL-6 content.

In recent years, it was found that both inflammatory mediators and pancreatic acinar cell apoptosis are related to AP[14,15]. Apoptosis participates in AP onset[16]. The apoptosis of pancreatic acinar cell might be a reaction beneficial to the body after the occurrence of pancreatitis[17,18]. Both necrosis and apoptosis are death modes of injured cells[19]. However, substantially different from necrosis, apoptosis will not release the harmful substance in lysosome or cause intense inflammatory reaction[20]. Necrosis prevails in SAP. The illness can be alleviated by apoptosis induction and aggravated by apoptosis inhibition[14]. In this experiment, according to the result of TUNEL staining, the apoptotic index was higher in treatment group than in model group ($P < 0.01$), and the pathological score was lower in treatment group than in model group ($P < 0.05$) at 6 h, demonstrating that dexamethasone can promote the apoptosis of pancreatic cells and protect pancreatic tissue.

The effect of glucocorticoid (represented by dexamethasone) on AP/SAP has been an issue in dispute. In 1952, Stephensen *et al*[21] for the first time reported the effect of glucocorticoid in AP treatment. Many empirical studies show glucocorticoid can improve the survival of AP animals[22,23] Its mechanisms mainly are: inhibiting the generation of inflammatory mediators and (or) inhibiting the effects of inflammatory mediators, enhancing body stress, improving microcirculation, alleviating endotoxemia, cleaning free radicals, inhibiting nitric oxide (NO) and expression of NF-κB, *etc*[24-26]. In terms of administration and dose, Dong *et al*[27,28] found a large dose of dexamethasone was obviously superior to the small dose dexamethasone in therapeutic effect and early use of dexamethasone was superior to dexamethasone of the same dose 5 h later. We used large doses of dexamethasone and achieved relatively sound therapeutic effects, obviously alleviating pathological changes of pancreas.

This empirical study used the improved Aho's method[29] to prepare SAP model, the rat survival of the dexamethasone treated group was significantly higher than that of the model group, but there was no marked difference between the two groups ($P > 0.05$). However, no matter gross or under light microscope, the treatment group has milder pancreatic tissue cell inflammatory pathological changes, less ascitic fluid and hemorrhage, and lower necrosis scope than the model group at all time points.

The contents of amylase and endotoxin in plasma and TNF-α, PLA_2 and IL-6 in serum were all lower in dexamethasone treated group than in model group. The apoptotic index was higher in treatment group than in model group while the inflammation, hemorrhage and necrosis of pancreas were all milder in treatment group than in model group, indicating that dexamethasone can improve the pancreatic injury of SAP rats by directly inducing pancreatic cell apoptosis, or indirectly inducing apoptosis through inhibition of excessive rise of TNF-α, IL-6, PLA_2, *etc*. In this experiment, no relation has been found between inflammatory mediators and pancreas apoptotic index. However, it has been found in many studies that the inflammatory mediators released by injured cells in AP can influence apoptosis. The role of inflammatory mediators that indirectly regulate apoptotic gene is significant and non-neglectable during its participation in apoptosis. It is worth mentioning that there are various but one influential factors act together to result in apoptosis during AP, presenting a network relation structure[30].

We used the tissue microarray section maker (Beecher Instruments, USA) to drill a hole 2.0 mm in diameter on recipient block and combined TUNEL staining method to examine the apoptotic index. The results indicate that the tissue chip 2.0 mm in diameter can achieve reliable experimental result, which is representative, time, energy and reagent saving, and convenient for control.

COMMENTS

Background

The severe acute pancreatitis (SAP) is one of the common acute abdomens in clinical practice. The pathogenesis of SAP is closely related to factors such as activation of pancreatin, release of inflammatory mediators, microcirculation disturbance and apoptosis. The recent studies prove the apoptosis could be a beneficial reaction to AP. The apoptosis of acinar cell in pancreas inducing injury can alleviate the inflammatory reaction. In this experiment, the mechanism of dexamethasone treatment in SAP was discussed and the changes of inflammatory mediator content and pancreatic acinar cell apoptosis were observed.

Research frontiers

To discuss the influence of dexamethasone on inflammatory mediators and apoptosis of rats with SAP, the authors established the rat SAP models and combined the tissue microarrays to observe the influence of dexamethasone on apoptosis of acinar cell in pancreas, providing a new theoretical basis for dexamethasone treatment of SAP and application of tissue microarrays in pancreatitis pathological examinations.

Innovations and breakthroughs

The tissue microarray has been applied to the pathohistological examination of pancreatitis to improve the study efficiency.

Applications

The sound therapeutic effects of a large dose of dexamethasone on SAP have been demonstrated. It is of some value to apply tissue microarrays to pathological examination and analysis of non-tumor diseases like pancreatitis.

Terminology

The apoptosis is a kind of self-protecting fashion namely the body starts the autogene program under certain pathological and physiological conditions and removes the irreparable cells, which is substantially different from necrosis. Tissue microarray (TMA), or tissue chip, is a method of harvesting small disks (diameter 0.6-2.0 mm) of tissue from a range of standard histologic sections and placing in an array on a recipient paraffin block by which hundreds of cases can be analyzed simultaneously. This technique allows maximization of tissue resources by analysis of small-core biopsies of blocks, rather than complete sections.

Peer review

Through animal studies, the authors investigated the influence of dexamethasone on inflammatory mediators and apoptosis of rats with severe acute pancreatitis. They concluded that the mechanism of dexamethasone treatment in acute pancreatitis is related to its inhibition of inflammatory mediator generation and induction of pancreatic acinar cell apoptosis.

REFERENCES

1 **Zhang XP**, Zhang L, Chen LJ, Cheng QH, Wang JM, Cai W, Shen HP, Cai J. Influence of dexamethasone on inflammatory mediators and NF-kappaB expression in multiple organs of rats with severe acute pancreatitis. *World J Gastroenterol* 2007; **13**: 548-556

2 **Zhang XP**, Zhang L, Yang P, Zhang RP, Cheng QH. Protective Effects of Baicalin and Octreotide on Multiple Organ Injury in Severe Acute Pancreatitis. *Dig Dis Sci* 2007 Jun 5; [Epub ahead of print]

3 **Wang ZF**, Pan CE, Liu SG. The role of inflammatory mediators in acute pancreatitis. *Shijie Huaren Xiaohua Zazhi* 1998, **6**: 170-171

4 **Gu J**, Li N. Acute pancreatitis and SIRS. *Gandan Waike Zazhi* 2004, **12**: 149-152

5 **Dugernier TL**, Laterre PF, Wittebole X, Roeseler J, Latinne D, Reynaert MS, Pugin J. Compartmentalization of the inflammatory response during acute pancreatitis: correlation with local and systemic complications. *Am J Respir Crit Care Med* 2003; **168**: 148-157

6 **Bentrem DJ**, Joehl RJ. Pancreas: healing response in critical illness. *Crit Care Med* 2003; **31**: S582-S589

7 **Yang J**, Murphy C, Denham W, Botchkina G, Tracey KJ, Norman J. Evidence of a central role for p38 map kinase induction of tumor necrosis factor alpha in pancreatitis-associated pulmonary injury. *Surgery* 1999; **126**: 216-222

8 **Kerekes L**, Antal-Szalmas P, Dezso B, Sipka S, Furka A, Miko I, Sapy P. In vitro examination of the influence of lipase and amylase on dog's pancreas tissue incubated with endotoxins, phospholipase A2 or cytokines. *Magy Seb* 2005; **58**: 120-124

9 **Norman JG**, Fink GW, Franz MG. Acute pancreatitis induces intrapancreatic tumor necrosis factor gene expression. *Arch Surg* 1995; **130**: 966-970

10 **Friess H**, Shrikhande S, Riesle E, Kashiwagi M, Baczako K, Zimmermann A, Uhl W, Buchler MW. Phospholipase A2 isoforms in acute pancreatitis. *Ann Surg* 2001; **233**: 204-212

11 **Caronna R**, Diana L, Nofroni I, Sibio S, Catinelli S, Sammartino P, Chirletti P. Effects of gabexate mesilate (FOY) on amylase and phospholipase A2 in human serum and pancreatic juice. *Dig Dis Sci* 2005; **50**: 868-873

12 **Caronna R**, Loretta D, Campedelli P, Catinelli S, Nofroni I, Sibio S, Sinibaldi G, Chirletti P. Gabexate mesilate (FOY) inhibition of amylase and phospholipase A(2) activity in sow pancreatic juice. *J Invest Surg* 2003; **16**: 345-351

13 **Bhatia M**. Novel therapeutic targets for acute pancreatitis and associated multiple organ dysfunction syndrome. *Curr Drug Targets Inflamm Allergy* 2002; **1**: 343-351

14 **Kaiser AM**, Saluja AK, Lu L, Yamanaka K, Yamaguchi Y, Steer ML. Effects of cycloheximide on pancreatic endonuclease activity, apoptosis, and severity of acute pancreatitis. *Am J Physiol* 1996; **271**: C982-C993

15 **Bhatia M**. Apoptosis of pancreatic acinar cells in acute pancreatitis: is it good or bad? *J Cell Mol Med* 2004; **8**: 402-409

16 **Wu ZJ**, Zhang YD, Lei ZM, Yu SH. Discuss apoptosis in pathogenesis of rat acute pancreatitis. *Zhongguo Xiandai Yixue Zazhi* 2003, **13**: 13-15

17 **Yasuda T**, Takeyama Y, Ueda T, Shinzeki M, Sawa H, Nakajima T, Kuroda Y. Breakdown of intestinal mucosa via accelerated apoptosis increases intestinal permeability in experimental severe acute pancreatitis. *J Surg Res* 2006; **135**: 18-26

18 **Chen HM**, Hsu JT, Chen JC, Ng CJ, Chiu DF, Chen MF. Delayed neutrophil apoptosis attenuated by melatonin in human acute pancreatitis. *Pancreas* 2005; **31**: 360-364

19 **McHugh P**, Turina M. Apoptosis and necrosis: a review for surgeons. *Surg Infect* (Larchmt) 2006; **7**: 53-68

20 **Samuilov VD**, Oleskin AV, Lagunova EM. Programmed cell death. *Biochemistry* (Mosc) 2000; **65**: 873-887

21 **Stephenson HE Jr**, Pfeffer RB, Saypol GM. Acute hemorrhagic pancreatitis; report of a case with cortisone treatment. *AMA Arch Surg* 1952; **65**: 307-308

22 **Sugiyama Y**, Kato S, Abe M, Mitsufuji S, Takeuchi K. Different effects of dexamethasone and the nitric oxide synthase inhibitor L-NAME on caerulein-induced rat acute pancreatitis, depending on the severity. *Inflammopharmacology* 2005; **13**: 291-301

23 **Paszt A**, Takacs T, Rakonczay Z, Kaszaki J, Wolfard A, Tiszlavicz L, Lazar G, Duda E, Szentpali K, Czako L, Boros M, Balogh A, Lazar G Jr. The role of the glucocorticoid-dependent mechanism in the progression of sodium taurocholate-induced acute pancreatitis in the rat. *Pancreas* 2004; **29**: 75-82

24 **Sugiyama Y**, Kato S, Abe M, Mitsufuji S, Takeuchi K. Different effects of dexamethasone and the nitric oxide synthase inhibitor L-NAME on caerulein-induced rat acute pancreatitis, depending on the severity. *Inflammopharmacology* 2005; **13**: 291-301

25 **Wang ZF**, Pan CE, Lu Y, Liu SG, Zhang GJ, Zhang XB. The role of inflammatory mediators in severe acute pancreatitis and regulation of glucocorticoids. *Hepatobiliary Pancreat Dis Int* 2003; **2**: 458-462

26 **Liu JS**, Wei XG, Fu J, Liu J, Yuan YZ, Wu YL. Stady of the relationship among endothelin,nitric oxide,oxgen free radical and acute pancreatitis. *Zhongguo Yishi Zazhi* 2003; **5**: 28-29

27 **Dong R**, Wang ZF, LV Y, Ma QJ. Treatment of severe acute pancreatitis with large dosage of dexamethsone in the earlier time. *Gandan Waike Zazhi* 2005; **13**: 58-60

28 **Dong R**, Wang ZF, Lu Y, Pan CE. Empirical study on severe acute pancreatitis treated by large dose of dexamethasone. *Zhonghua Putong Waike Zahi* 2001; **10**: 314-317

29 **Aho HJ**. Experimental pancreatitis in the rats: sodium taurocholate-induced acute hemorrhagic pancreatitis. *Scand J Gastroenterol* 1980; **15**: 411-416

30 **Zhang XP**, Lin Q. Advancement of study on the relationship between mediators of inflammation and apoptosis in acute pancreatitis. *Shijie Huaren Xiaohua Zazhi* 2005; **13**: 2773-2777

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CASE REPORT

Histological changes at an endosonography-guided biliary drainage site: A case report

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Abstract

Endosonography-guided biliary drainage (ESBD) is a new method enabling internal drainage of an obstructed bile duct. However, the histological conditions associated with fistula development *via* the duodenum to the bile duct have not been reported. We performed ESBD 14 d preoperatively in a patient with an ampullary carcinoma and histologically confirmed changes in and around the fistula. The female patient developed no complications relevant to ESBD. Levels of serum bilirubin and hepatobiliary enzymes declined quickly, and pancreatoduodenectomy was carried out uneventfully. The resected specimen was sliced and stained with hematoxylin-eosin. Histological evaluation of the puncture site in the duodenum and bile-duct wall, and the sinus tract revealed no hematoma, bile leakage, or abscess in or around the sinus tract. Little sign of granulation, fibrosis, and inflammatory cell infiltration was observed. Although further large-scale confirmatory studies are needed, the findings here may encourage more active use of ESBD as a substitute for percutaneous transhepatic drainage in cases with failed/difficult endoscopic biliary stenting.

Key words: Endosonography; Endoscopic ultrasound-guided fine needle aspiration; Endoscopic biliary drainage; Biliary stenting; Endoscopic retrograde cholang iopancreatography; Obstructive jaundice; Biliary stricture

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INTRODUCTION

The role of endosonography (ES) in digestive diseases is gradually expanding from diagnostic to therapeutic applications. In the mid 1990s, the feasibility of ES-guided cholangiopancreatography was first reported by Harada *et al*[1] (pancreatography) and Wiersema *et al*[2] (cholangiography). Several reports on the application of this technique for therapeutic purposes, such as ES as a guide for biliary drainage, have been published.

We have recently applied ES-guided biliary drainage (ESBD) for preoperative decompression of the biliary tree in a patient with cancer of the papilla of Vater. The results of histological evaluation of and around the sinus tract are reported herein.

CASE REPORT

A 76-year-old Japanese woman was admitted to our department, complaining of jaundice and itching. Laboratory data on admission showed the following abnormalities: serum total bilirubin, 11.7 mg/dL; glutamic oxaloacetic transaminase (GOT), 389 IU/L; glutamic pyruvic transaminase (GPT), 285 IU/L; and alkaline phosphatase (ALP), 1487 IU/L. Transabdominal ultrasonography and abdominal computed tomography revealed a mass in the ampullary region, along with dilatation of the bile and pancreatic ducts (Figure 1). To resolve her complaints, endoscopic retrograde cholangiopancreatography (ERCP) with biliary stenting was attempted. However, cannulation of the bile duct was unsuccessful because stenosis of the descending portion of the duodenum, and the presence of a tumor at the papilla of Vater that bled easily when contacted, made it impossible to manipulate the endoscope to identify the orifice. After obtaining written informed consent, ESBD was undertaken. Following visualization of the extrahepatic bile duct with a curved linear array echoendoscope (GF-UC240P; Olympus, Tokyo, Japan), the dilated bile duct was punctured *via* the upper part of the descending portion of the duodenum with a 19G needle (Olympus) (Figure 2A). Following removal of the core needle, white bile was aspirated. A small amount of contrast agent was then injected *via* the sheath catheter into the bile duct, to guide stent placement and to confirm the absence of bile leakage or extravasation. A guidewire 0.889 mm in diameter (Jagwire; Boston Scientific, Natik, MA, USA) was introduced into the sheath catheter and inserted into the intrahepatic bile duct. Subsequent to

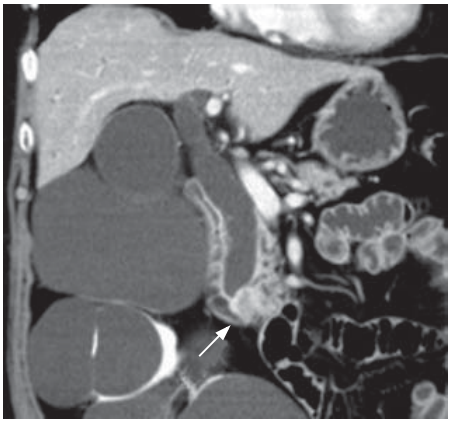

Figure 1 CT reveals a mass in the ampullary region (arrow) with a dilated extrahepatic bile duct. The patient also had multiple renal cysts.

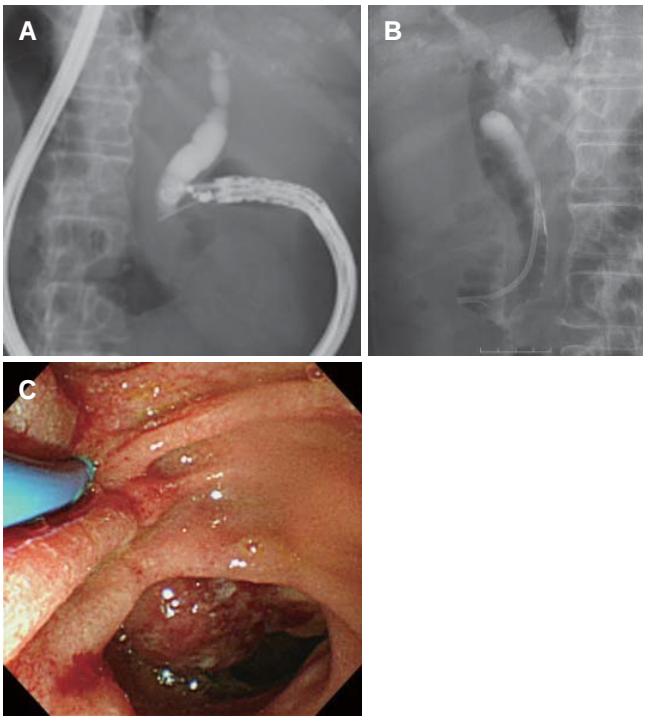

Figure 2 A, B: Puncture of the bile duct via the duodenum under endosonographic guidance, followed by deployment of a plastic stent. **C:** Endoscopic view after stent placement.

removing the sheath catheter and leaving the guidewire *in situ*, we dilated the puncture tract with a dilator catheter 7F in diameter, and placed a 7F plastic stent (Figure 2B and C). The patient developed no symptoms related to the procedure and her initial complaints also soon disappeared. One week after the procedure, levels of serum total bilirubin, GOT, GPT, and ALP had declined to 3.2 mg/dL, 33 IU/L, 47 IU/L, and 780 IU/L, respectively. She underwent pancreaticoduodenectomy 14 d after ESBD. Macroscopically, the sites of the puncture in the bile duct and duodenum were clear without infection, hemorrhage or hematoma (Figure 3).Histological examination revealed mild inflammatory cell infiltrate adjacent to the sinus tract in the duodenal and bile duct walls, without hemorrhage (Figure 4). A fistula was formed

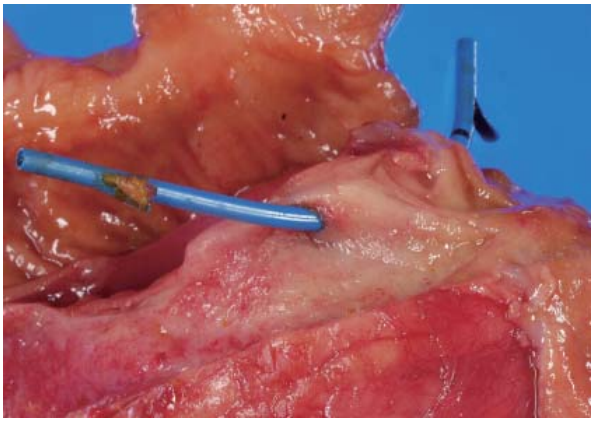

Figure 3 Fresh resected specimen. No hematoma or abscess is seen at the site of the puncture in the bile duct.

along the tract of the puncture without significant reactive changes. No evidence of severe inflammation, such as bile peritonitis, was found on the extraluminal side of either the duodenum or the bile duct.

DISCUSSION

The role of ES in the management of gastrointestinal diseases has evolved from imaging of the gut wall and organs adjacent to the alimentary tract, to its use as a guide for tissue sampling with a fine-needle, as well as in therapeutic applications such as injection of agents into tumors and drainage of pancreatic pseudocysts. Endosonographic approaches to an inaccessible bile duct and pancreatic duct by ERCP were first reported in the mid 1990s. In 1996, Wiersema *et al*[2] reported the feasibility of ES-guided cholangiopancreatography. Subsequently, several case series have been published on the feasibility and effectiveness of ESBD, the first being by Giovannini *et al*[3] in 2001, in a pancreatic cancer patient with a history of failed cannulation of the bile duct, even after precutting, who underwent preoperative chemoradiotherapy. They succeeded in the deployment of a 10F stent in a two-step procedure by changing endoscopes. In 2003, Burmester *et al*[4] reported three cases of successful stent placement among four attempts at ESBD. They achieved biliary drainage by a one-step technique, a modification of the Seifert technique[9] for drainage of pancreatic pseudocysts. They approached the bile duct via the duodenum, stomach and even the jejunum. Mallery *et al*[5] performed endoscopic ultrasound-guided rendezvous drainage of the bile duct and pancreatic duct after unsuccessful ERCP. They succeeded in stent placement following antegrade traverse of the stenosis with a guidewire in three (two biliary and one pancreatic) of six cases. Puspok *et al*[6] applied this technique to cases of bile duct stones with difficult cannulation of the bile duct, and reported excellent results. Kahaleh *et al*[7] recently published their experience of ESBD in 23 patients. They carried out puncture of the bile duct *via* the stomach, duodenum and jejunum, and concluded that intrahepatic access to the biliary system appears safer than the extrahepatic approach.

As described here, acceptable success rates and inci-

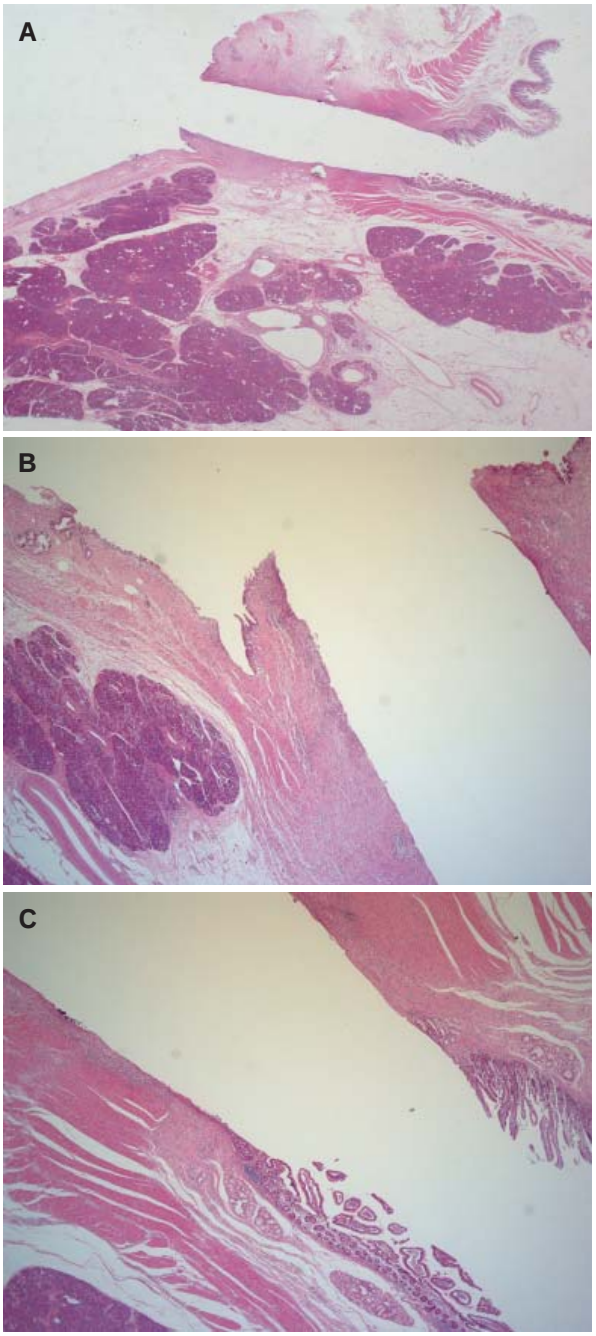

Figure 4 Microscopic views of the sinus tract. Mild inflammatory cell infiltrate adjacent to the sinus tract in the duodenal wall and the bile duct wall is seen, without hemorrhage or abscess formation. A fistula is formed along the tract of the puncture but without significant reactive changes. **A:** Low-power view of the sinus tract (× 1.25); **B:** End of the sinus tract on the bile duct side (× 5); **C:** End of the sinus tract on the duodenal side (× 5).

dence of complications were reported for puncture and stent placement under sonographic guidance. However, there have been no reports of the influence of this technique on the gut wall, bile duct, or intervening tissues between them. This is believed to be the first report describing the preoperative performance of ESBD and the histological condition of the sinus tract established by this method. The duodenum, bile duct and sinus tract showed no adverse histological changes. These results are attributable to the use of endosonography as a guide. This technique has a low potential risk of major bleeding as color Doppler evaluation is utilized for determining the route of the puncture. Injury to adjacent organs is also minimized due to clear visualization of the structures in the area of interest, with the use of high-frequency ultrasound. Some authors[4,6,8] have applied a fistulotome with electrocautery for puncture. However, it seems theoretically safer to apply a simple puncture needle to avoid damage to the tissues in and around the pathway of the puncture.

The present study elucidated histological changes at and around the sinus tract, following ESBD. The results that no severe inflammation or hemorrhage occurred should encourage the wider use of this technique, although further large-scale studies will be needed.

In general, the method of choice for biliary obstruction is endoscopic biliary stenting. Percutaneous transhepatic cholangio-drainage (PTCD) is considered a substitute. However, PTCD can result in pain after placement of the drainage tube, and can restrict activities of daily living. In contrast, ESBD is a safe and effective method for biliary drainage and does not cause pain or restriction of daily living, as is the case with endoscopic biliary stenting. It should therefore replace PTCD in a large proportion of those patients with an obstructed biliary tree and with difficult cannulation of the bile duct, duodenal stenosis, or deformity of the papilla of Vater caused by cancer, which hinders detection of the orifice, regardless of the likelihood of successful PTCD.

As is the case with plastic stents in endoscopic biliary stenting, the diameter of the stent available in ESBD is restricted by the diameter of the working channel of the endoscope employed. The endoscope we used had a 2.8-mm diameter working channel, allowing the use of only 7F stents, or smaller.

Dilation of the sinus tract can be achieved with a dilator balloon, following insertion of the guidewire into the bile duct *via* the lumen of the placed stent and removal of the sent alone. Therefore, once access to the bile duct has been established, it is possible to place a stent with a larger caliber, even a metallic stent, without difficulty in either a one- or two-step procedure. In the long term, it is inevitable that plastic stents will become occluded. Deployment of a metallic stent will prolong patency, as shown in endoscopic transpapillary biliary stenting[10]. Covered metallic stents are deemed to be advantageous in avoiding bile leakage.

Further development of accessory devices specialized for ESBD will help expand its indications.

REFERENCES

1 **Harada N**, Kouzu T, Arima M, Asano T, Kikuchi T, Isono K. Endoscopic ultrasound-guided pancreatography: a case report. *Endoscopy* 1995; **27**: 612-615
2 **Wiersema MJ**, Sandusky D, Carr R, Wiersema LM, Erdel WC, Frederick PK. Endosonography-guided cholangiopancreatography. *Gastrointest Endosc* 1996; **43**: 102-106
3 **Giovannini M**, Moutardier V, Pesenti C, Bories E, Lelong B, Delpero JR. Endoscopic ultrasound-guided bilioduodenal anastomosis: a new technique for biliary drainage. *Endoscopy* 2001; **33**: 898-900
4 **Burmester E**, Niehaus J, Leineweber T, Huetteroth T. EUS-cholangio-drainage of the bile duct: report of 4 cases. *Gastrointest Endosc* 2003; **57**: 246-251

5 **Mallery S**, Matlock J, Freeman ML. EUS-guided rendezvous drainage of obstructed biliary and pancreatic ducts: Report of 6 cases. *Gastrointest Endosc* 2004; **59**: 100-107
6 **Puspok A**, Lomoschitz F, Dejaco C, Hejna M, Sautner T, Gangl A. Endoscopic ultrasound guided therapy of benign and malignant biliary obstruction: a case series. *Am J Gastroenterol* 2005; **100**: 1743-1747
7 **Kahaleh M**, Hernandez AJ, Tokar J, Adams RB, Shami VM, Yeaton P. Interventional EUS-guided cholangiography: evaluation of a technique in evolution. *Gastrointest Endosc* 2006; **64**: 52-59
8 **Yamao K**, Sawaki A, Takahashi K, Imaoka H, Ashida R, Mizuno N. EUS-guided choledochoduodenostomy for palliative biliary drainage in case of papillary obstruction: report of 2 cases. *Gastrointest Endosc* 2006; **64**: 663-667
9 **Seifert H**, Faust D, Schmitt T, Dietrich C, Caspary W, Wehrmann T. Transmural drainage of cystic peripancreatic lesions with a new large-channel echo endoscope. *Endoscopy* 2001; **33**: 1022-1026
10 **Isayama H**, Komatsu Y, Tsujino T, Sasahira N, Hirano K, Toda N, Nakai Y, Yamamoto N, Tada M, Yoshida H, Shiratori Y, Kawabe T, Omata M. A prospective randomised study of "covered" versus "uncovered" diamond stents for the management of distal malignant biliary obstruction. *Gut* 2004; **53**: 729-734

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CASE REPORT

Synchronous isolated splenic metastasis from colon carcinoma and concomitant splenic abscess: A case report and review of the literature

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Abstract

This study aimed to describe a case in which an isolated splenic metastasis was synchronous with the colonic primary and a concomitant splenic abscess was associated. A wide review of the literature was also performed. A 54-year-old woman with abdominal pain and fever was admitted to our department. Abdominal CT revealed two low-density areas in the spleen and wall-thickening of the left colonic flexure, which was indistinguishable from the spleen parenchyma. The patient underwent emergency celiotomy, with the presumptive diagnosis of obstructing colon carcinoma of the splenic flexure, and concomitant splenic abscess. Subtotal colectomy and splenectomy were performed. Pathological findings were consistent with mucinous colonic carcinoma, synchronous isolated splenic metastasis and concomitant splenic abscess. This paper is also a review of the existing literature on the association between colorectal cancer and splenic metastasis. Only 41 cases of isolated splenic metastasis from colon carcinoma have been reported in the literature. This report is the third described case of synchronous isolated splenic metastasis from colon carcinoma. Only one case with concomitant splenic abscess has been previously reported. When obstructing left-sided colorectal cancer is suspected, careful CT examination can allow early diagnosis of splenic involvement by the tumor. The literature review suggests that there might be a significant improvement in survival following splenectomy for a metachronous isolated splenic metastasis from colon carcinoma. Prognosis for synchronous splenic metastasis seems to be related to the advanced stage of the disease. Nevertheless, no definitive conclusions can be drawn because of the small number of cases.

INTRODUCTION

Primary and metastatic tumors of the spleen are described as unusual[1], excluding secondary involvement by lymphoma[2]. Since metastatic carcinoma involving the spleen is usually a manifestation of widely disseminated disease, isolated splenic metastasis from colorectal carcinoma is not a common occurrence[1,3]. Its rareness has been hypothetically explained by several characteristics of the spleen, such as anatomical, histological and immunological features[4]. Most cases are asymptomatic and the diagnosis is usually made by imaging studies during the diagnostic work up for colon cancer[5]. However, a few patients become exceptionally symptomatic following spontaneous rupture of the spleen, or the presence of an associated splenic abscess[6,7].

We report the case of a synchronous isolated splenic metastasis from colonic carcinoma, with a concomitant splenic abscess, and we also review all cases of isolated splenic metastasis from colorectal cancer reported in the literature. To the best of our knowledge, only one case of splenic metastasis from colonic carcinoma associated with concomitant splenic abscess has been reported in the literature[7], which is an extremely rare clinical entity.

CASE REPORT

In June 2006, a 54-year-old Caucasian woman was referred to our emergency department because of abdominal pain associated with intermittent fever over 40℃, shaking and chills. She also complained of general fatigue and loss of appetite. Otherwise, her previous medical history was unremarkable. On clinical examination, the patient was pale and shocked. Blood pressure and pulse rate were 90/60 mmHg and 98/min, respectively. The abdomen was distended, with tenderness in the left hypochondrium. There

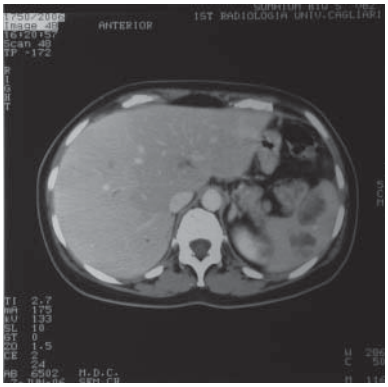

Figure 1 Two low density areas in the spleen (Axial CT-scan).

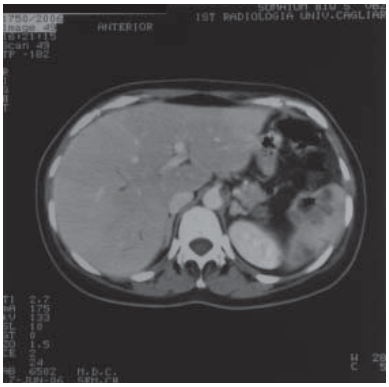

Figure 2 Wall thickening of the left flexure of the colon indistinguishable from the spleen parenchyma (Axial CT-scan).

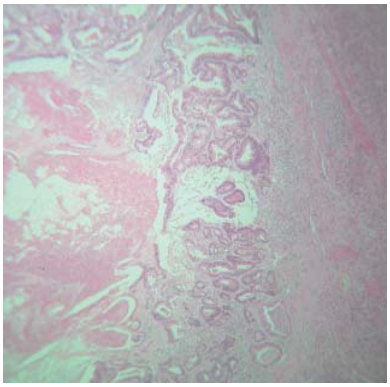

Figure 3 Splenic tumor showing glandular pattern consistent with metastasis from colonic mucinous carcinoma (HE, x 40).

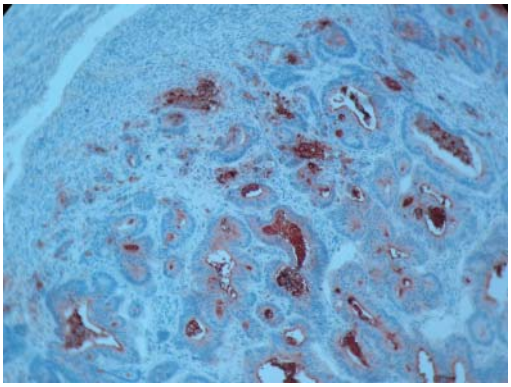

Figure 4 CEA along the luminal border of tumor cells infiltrating splenic pulp (anti-CEA monoclonal antibody staining, x 100).

was neither hepatosplenomegaly nor lymphadenopathy. Sepsis was identified both by a clear clinical picture which showed high fever and hemodynamic instability, and by positive blood culture which grew *Escherichia coli* and *Bacteroides fragilis*. Laboratory data on hospital admission were as follows: white blood cell count, 148 × 10^9/L; red blood cell count, 280 × 10^{12}/μL; hemoglobin, 67 g/L; thrombocyte count 383× 10^9/L; fibrinogen, 5.17 g/L; albumin 23 ug/L; and carcinoembryonic antigen (CEA), 31.1 ug/L (normal range < 2.50 ug/L).

Chest X-ray revealed a left pleural effusion. Abdominal plain radiography showed intestinal obstruction with typical air fluid levels in the bowel. Abdominal ultrasonography demonstrated a hypoechoic area with unclear margins in the lower pole of the spleen, with the features of a splenic abscess. Enhanced abdominal CT revealed two low-density areas in the spleen (Figure 1) and wall-thickening of the left colonic flexure, which was indistinguishable from the spleen parenchyma (Figure 2). Echocardiography was normal and detected no vegetations. The patient was treated with fluid, intensive antibiotics and blood transfusion as initial therapy, and then she underwent an emergency operation for a presumptive diagnosis of obstructing colonic carcinoma and septic shock from a concomitant splenic abscess. On explorative celiotomy, the splenic flexure of the colon presented a mass occluding the lumen, infiltrating the entire colonic wall, and invading the lower pole of the spleen. There were neither hepatic metastases nor peritoneal dissemination. Frozen section examination of the spleen was performed after splenectomy. Frozen section showed the presence of splenic metastasis from adenocarcinoma, with a concomitant splenic abscess. A subtotal colectomy with side-to-side ileo-sigmoid anastomosis was then performed. The spleen weighed 160 g and measured 12 cm × 7 cm × 4.5 cm. On gross examination, the tumor originated from the left colonic flexure and invaded the spleen, in which it formed a fistula and an abscess in the metastatic tissue. The splenic metastasis measured 4.5 cm at its largest diameter and had a central abscess with a cavity of 2 cm. Histopathological findings were consistent with mucinous adenocarcinoma of the colon, synchronous isolated splenic metastasis from the primary colonic tumor (Figure 3), and concomitant splenic abscess, without metastatic lymph-node involvement (T4N0M1). Immunohistochemistry of both colonic carcinoma and splenic metastasis was performed using anti-CEA monoclonal antibody (Clone Ⅱ-7, Dako Corporation, Carpinteria, CA, USA). Staining of CEA along the luminal border of tumor cells was demonstrated both in the colonic carcinoma and in the metastasis that infiltrated the splenic pulp (Figure 4). Left pleural effusion persisted for 10 d after the operation, and the patient was finally discharged on postoperative day 15.

After 1 mo, the CEA level dropped to 3.0 μg/L. The patient was treated with adjuvant chemotherapy. Six months after the operation, CEA level was 10.4 μg/L. Abdominal CT and positron emission tomography (PET) revealed a solitary liver metastasis of 2 cm, which was surgically removed. Exploration of the abdominal cavity revealed no further evidence of neoplastic disease. Afterward, the patient was once more subjected to adjuvant chemotherapy.

Table 1 Literature review of isolated splenic metastasis from colorectal carcinoma

Year	Author	Journal	Site of primary tumor	Synchronous metastasis	Metachronous metastasis	Concomitant splenic abscess
1969	Dunbar[10]	Mayo Clinic Proc	Rectum		1	
1982	Waller[25]	Clin Nucl Med	Sigmoid colon		1	
1986	Slavin[26]	Clin Nucl Med	Right colon		1	
1992	Capizzi[27]	South Med J	Rectum		1	
1993	Thomas[28]	Eur J Surg Oncol	Left colon		1	
1997	Mainprize[3]	Br J Surg	Splenic flexure		1	
1997	Indudhara[13]	South Med J	Sigmoid colon		1	
1999	Achuthan[6]	Ann R Coll Surg Engl	Rectum		1	
1999	Weathers[24]	Dis Colon Rectum[1]	Sigmoid colon		1	
1999	Vadalà[29]	Minerva Chir	Left colon		1	
2000	Kim[4]	J Korean Med Sci	Right colon		1	
2000	Lee[30]	Am Surg	Not specified		1	
2001	Place[1]	Am Surg	Sigmoid colon		1	
2001	Avesani[11]	Am J Clin Oncol	Left colon	1		
2001	Paramelle[7]	J Radiol	Left colon	1		1
2001	Okuyama[20]	Jpn J Clin Oncol	Sigmoid colon		1	
2001	Quoted in Okuyama[20]	Jpn J Clin Oncol[2]	Left colon		11	
			Left + right colon		1	
			Right colon		7	
			Rectum		1	
2003	Genna[17]	Minerva Chir	Left colon		1	
2004	Cavallaro[12]	J Exp Clin Cancer Res	Sigmoid colon		1	
2004	Pizzirusso[31]	Acta Chir Belg	Left colon		1	
2006	Cabanas[16]	Tumori	Sigmoid colon		1	
2006	Gencosmanoglu[5]	World J Surg Oncol	Sigmoid colon + splenic flexure		1	
2007	Pisanu	Present report	Splenic flexure	1		1
			Total	3	39	2

[1]This metastasis occurred 3 mo after colonic operation; [2]From the review of the Japanese literature.

DISCUSSION

Approximately 20% of colorectal carcinomas are metastatic at their clinical presentation[8]. Metastases to other sites in the absence of liver, lung or axial skeleton involvement are very rare[1,9]. Similar to the results of an autopsy study by Berge, microscopic splenic metastases were found in 7%-34% of cancer patients[2]. The same author reported the incidence of splenic micrometastases arising from colorectal carcinoma to be as high as 2% in 1019 colorectal tumors, but all of these involved other organs as well[2]. In 1969 Dunbar *et al*[10] published the first case report of isolated splenic metastasis from colorectal carcinoma and to date, only 41 such cases have been reported in the literature (Table 1). All but two of previously described cases of solitary splenic metastases from colon carcinoma had a metachronous metastasis (Table 1). We have described the third reported case in which an isolated splenic lesion was synchronous with colonic carcinoma[7,11]. The particularly interesting aspect of our case was also related to the simultaneous presence of a splenic abscess, because the only other reported case with similar clinical and pathological features is that by Paramelle *et al*[7].

The rareness of splenic metastasis arising from colonic carcinoma suggests the existence of some mechanism that prohibits tumor cell proliferation in the spleen. Anatomical and immunological characteristics may be reasons for the rarity of isolated splenic metastasis[4]. From an anatomical perspective, the sharp angle of the splenic artery with the celiac axis and rhythmic contraction by the sinusoidal splenic architecture are limiting factors for metastasis[4,12]. According to Indudhara *et al*[13], neoplastic cells can reach the splenic vein and parenchyma by retrograde diffusion through the inferior mesenteric vein. The spleen parenchyma contains no afferent lymphatic vessels, but they are present in the capsular, subcapsular and trabecular regions[12]. Tumor cells might also reach the spleen *via* the lymphatic system, which explains the typical subcapsular localization of isolated splenic metastases[12]. As the spleen is the second largest organ of the reticuloendothelial system, immune surveillance appears to potently inhibit tumor cell proliferation[14]. Moreover, experimental studies have shown that the growth rate of adenocarcinoma cells injected into the spleen is significantly lower than that of the same cells injected into the liver[15].

Histopathological findings in our case were consistent with mucinous adenocarcinoma of the colon, as in three other cases of isolated splenic metastasis[3,16,17]. Mucinous gastrointestinal malignancies are thought to cause perforation and infiltration of the full thickness of the bowel wall, which lead to extensive invasion of the pericolic fat[3,16]. A new mechanism has been proposed in the case of contiguous splenic metastasis from mucinous colonic tumors. Cabanas *et al*[16] have suggested that mucus-producing epithelial cells become trapped within the trabecula of the splenic capsule, in congenital clefts of the spleen, or in microfissures caused by trauma. The resistance of the splenic capsule or fibrous tissue surrounding the spleen causes the mucinous tumor to expand into the soft splenic parenchyma, rather than

the peritoneal cavity[16]. Following the more aggressive behavior of mucinous colonic tumors, this mechanism may have explained the presence of the synchronous splenic metastasis in our case. Furthermore, since CEA appears as an immunosuppressant, and acts as an adhesion molecule between tumor cells and visceral macrophages, colonic tumor cells with positive CEA staining should display more aggressive behavior[4,18]. In regard to these biological functions, CEA expression might be associated with the occurrence of isolated splenic metastasis[4] (Figure 4).

The diagnosis of isolated splenic metastasis is generally made by imaging studies during the diagnostic work up for colonic cancer[5]. Only a few patients with splenic metastasis become symptomatic because of the presence of an associated splenic abscess[8] or spontaneous rupture of the spleen[6,19], as in our case. Okuyama *et al*[20] have pointed out that only six of 28 reported patients were symptomatic at the time of diagnosis. Our patient became symptomatic as a result of abdominal occlusion and sepsis originating from the splenic abscess, in which *E. coli* and *B. fragilis* grew, as in most cases of colonic abscess associated with colonic cancer[21]. The association between splenic abscess and colonic cancer is a very rare clinical entity[22], as is isolated splenic metastasis. Only a few cases have been reported in the literature, and sometimes the splenic abscess resulted from a direct fistula of descending colon carcinoma, without spleen metastasis[23]. The most frequent complication of splenic abscess is its rupture into the peritoneal cavity, and untreated splenic abscesses have a high mortality rate[23].

Most previously described patients with solitary splenic metastasis from colon carcinoma had a disease-free survival of 3-144 mo after the primary tumor[11,17,24]. Long-term survival after splenectomy in patients with isolated metachronous splenic metastasis from colon carcinoma varied from 0.5 to 7 years[11,20,24]. As a result, prognosis of isolated splenic metastasis after splenectomy appears to be rather optimistic, despite the fact that splenic metastasis is one form of distant metastasis[20]. In our case of synchronous metastasis, intensive follow-up revealed a solitary liver metastasis 6 mo after operation, without further evidence of neoplastic disease in the abdominal cavity. However, the disease-free interval after splenectomy in the case of synchronous splenic metastasis reported by Avesani *et al*[11] was 10 mo, and the patients died of diffuse carcinomatosis after 1 year.

The spleen is considered unfavorable to the development of metastases but the reason for this is not clearly understood. An isolated splenic metastasis from colon carcinoma is a rare clinical finding. On the basis of the present case, when an obstructing left-sided colorectal cancer is suspected in emergency setting, careful examination of the abdominal CT-scan can allow early diagnosis of a splenic involvement by the tumor. Clinicians must pay close attention to the spleen for the early diagnosis of isolated splenic metastasis when routinely evaluating abdominal CT-scan and abdominal ultrasonography following curative resection of primary colorectal cancer. Splenectomy is necessary in the presence of isolated metastases from colon carcinoma both synchronous and methacronous. The occurrence of a splenic abscess makes emergency splenectomy mandatory as the most frequent complication is its rupture into the peritoneal cavity.

Splenectomy followed by chemotherapy seems to be the preferred treatment of isolated splenic metastases from colorectal carcinoma. There are few data available about recurrence after splenectomy for metastases of this type. Literature review suggests that there might be a significant improvement of long-term survival following splenectomy for methacronous splenic metastasis arising from colon carcinoma. Prognosis for synchronous splenic metastasis seems to be related to the advanced stage of the disease. Nevertheless, following the small number of cases reported in the literature, no definitive conclusions can be drawn.

REFERENCES

1 **Place RJ**. Isolated colon cancer metastasis to the spleen. *Am Surg* 2001; **67**: 454-457
2 **Berge T**. Splenic metastases. Frequencies and patterns. *Acta Pathol Microbiol Scand [A]* 1974; **82**: 499-506
3 **Mainprize KS**, Berry AR. Solitary splenic metastasis from colorectal carcinoma. *Br J Surg* 1997; **84**: 70
4 **Kim JC**, Jeong CS, Kim HC, Yu CS, Kang GH, Lee MG. Isolated splenic metastasis from colorectal carcinoma: a case report. *J Korean Med Sci* 2000; **15**: 355-358
5 **Gencosmanoglu R**, Aker F, Kir G, Tozun N. Isolated metachronous splenic metastasis from synchronous colon cancer. *World J Surg Oncol* 2006; **4**: 42
6 **Achuthan R**, Joseph A, Haray PN. Splenic metastasis from a rectal tumour: an unusual presentation. *Ann R Coll Surg Engl* 1999; **81**: 139
7 **Paramelle PJ**, Ferretti G, Desroches E, Coulomb M. Quid? Cancer of the left colonic angle with colonic-splenic fistula, thrombosis of the splenic vein and left portal branch. *J Radiol* 2001; **82**: 511-513
8 **Pedersen IK**, Burcharth F, Roikjaer O, Baden H. Resection of liver metastases from colorectal cancer. Indications and results. *Dis Colon Rectum* 1994; **37**: 1078-1082
9 **Klein B**, Stein M, Kuten A, Steiner M, Barshalom D, Robinson E, Gal D. Splenomegaly and solitary spleen metastasis in solid tumors. *Cancer* 1987; **60**: 100-102
10 **Dunbar WH**, Beahrs OH, Morlock CG. Solitary splenic metastasis incidental to rectal carcinoma: report of a case. *Mayo Clin Proc* 1969; **44**: 40-45
11 **Avesani EC**, Cioffi U, De Simone M, Botti F, Carrara A, Ferrero S. Synchronous isolated splenic metastasis from colon carcinoma. *Am J Clin Oncol* 2001; **24**: 311-312
12 **Cavallaro A**, Modugno P, Specchia M, Pontenza AE, Loschiavo V, Colli R, Lauriola L, Barone C. Isolated splenic metastasis from colon cancer. *J Exp Clin Cancer Res* 2004; **23**: 143-146
13 **Indudhara R**, Vogt D, Levin HS, Church J. Isolated splenic metastases from colon cancer. *South Med J* 1997; **90**: 633-636
14 **Gabizon A**, Small M, Trainin N. Kinetics of the response of spleen cells from tumor-bearing animals in an in vivo tumor neutralization assay. *Int J Cancer* 1976; **18**: 813-819
15 **Miller JN**, Milton GW. An experimental comparison between tumour growth in the spleen and liver. *J Pathol Bacteriol* 1965; **90**: 515-521
16 **Cabanas J**, Gomes da Silva R, Zappa L, Esquivel J, Cerruto C, Goldstein P, Sugarbaker PH. Splenic metastases from mucinous neoplasms of the appendix and colon. *Tumori* 2006; **92**: 104-112
17 **Genna M**, Leopardi F, Valloncini E, Molfetta M, De Manzoni G, Castelli A. Metachronus splenic metastasis of colon cancer. A case report. *Minerva Chir* 2003; **58**: 811-814

18 **Kim JC**, Han MS, Lee HK, Kim WS, Park SK, Park KC, Bodmer WF, Rowan AJ, Kim OJ. Distribution of carcinoembryonic antigen and biologic behavior in colorectal carcinoma. *Dis Colon Rectum* 1999; **42**: 640-648
19 **al-Obaidi SM**. Spontaneous rupture of the spleen due to metastatic carcinoma. *Br J Clin Pract* 1989; **43**: 385-386
20 **Okuyama T**, Oya M, Ishikawa H. Isolated splenic metastasis of sigmoid colon cancer: a case report. *Jpn J Clin Oncol* 2001; 31: 341-345
21 **Namavar F**, Theunissen EB, Verweij-Van Vught AM, Peerbooms PG, Bal M, Hoitsma HF, MacLaren DM. Epidemiology of the Bacteroides fragilis group in the colonic flora in 10 patients with colonic cancer. *J Med Microbiol* 1989; **29**: 171-176
22 **Belinkie SA**, Narayanan NC, Russell JC, Becker DR. Splenic abscess associated with Streptococcus bovis septicemia and neoplastic lesions of the colon. *Dis Colon Rectum* 1983; **26**: 823-824
23 **Kawamoto K**, Teramoto T, Watanabe M, Kase S, Shatari T, Hasegawa H, Fujita S, Kuo TH, Kawano Y, Kitajima M. Splenic abscess associated with colon cancer: a case report. *Jpn J Clin Oncol* 1993; **23**: 384-388
24 **Weathers BK**, Modesto VL, Gordon D. Isolated splenic metastasis from colorectal carcinoma: report of a case and review of the literature. *Dis Colon Rectum* 1999; **42**: 1345-1348
25 **Waller RM 3rd**, Fajman WA. An unusual cause of an isolated, focal splenic defect demonstrated by liver-spleen scintigraphy. *Clin Nucl Med* 1982; **7**: 5-7
26 **Slavin JD Jr**, Mathews J, Spencer RP. Splenectomy for splenic metastasis from carcinoma of colon. *Clin Nucl Med* 1986; **11**: 491-492
27 **Capizzi PJ**, Allen KB, Amerson JR, Skandalakis JE. Isolated splenic metastasis from rectal carcinoma. *South Med J* 1992; **85**: 1003-1005
28 **Thomas SM**, Fitzgerald JB, Pollock RE, Evans DB. Isolated splenic metastases from colon carcinoma. *Eur J Surg Oncol* 1993; **19**: 485-490
29 **Vadala G**, Caragliano L, Castorina R, Caragliano V, Caragliano P. Splenic metastases. *Minerva Chir* 1999; **54**: 273-276
30 **Lee SS**, Morgenstern L, Phillips EH, Hiatt JR, Margulies DR. Splenectomy for splenic metastases: a changing clinical spectrum. *Am Surg* 2000; **66**: 837-840
31 **Pizzirusso F**, Gillet JP, Fobe D. Isolated spleen metastatic involvement from a colorectal adenocarcinoma complicated with a gastrosplenic fistula. A case report and literature review. *Acta Chir Belg* 2004; **104**: 214-216

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CASE REPORT

Benign retroperitoneal schwannoma presenting as colitis: A case report

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Abstract

We report a case of a patient presenting with clinical, radiological and endoscopic features of colitis due to a compressive left para-aortic mass. Total open surgical excision was performed, which resulted in complete resolution of colitis. Histopathology and immunohistochemistry revealed benign retroperitoneal schwannoma. These neural sheath tumors rarely occur in the retroperitoneum. They are usually asymptomatic but as they enlarge they may compress adjacent structures, which leads to a wide spectrum of non-specific symptoms, including lumbar pain, headache, secondary hypertension, abdominal pain and renal colicky pain. CT and MR findings show characteristic features, but none are specific. Schwannoma can be isolated sporadic lesions, or associated with schwannomatosis or neurofibromatosis type Ⅱ (NF2). Although they vary in biological and clinical behavior, their presence is, in nearly every case, due to alterations or absence of the *NF2* gene, which is involved in the growth regulation of Schwann cells. Both conditions were excluded by thorough mutation analysis. Diagnosis is based on histopathological examination and immunohistochemistry. Total excision is therapeutic and has a good prognosis. Schwannomatosis and NF2 should be excluded through clinical diagnostic criteria. Genetic testing of NF2 is probably not justified in the presence of a solitary retroperitoneal schwannoma.

Key words: Colitis; Neurofibromatosis; Retroperitoneum; Schwannoma

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INTRODUCTION

Schwannomas or neurilemmomas are encapsulated tumors arising from the neural sheath of peripheral nerves. They are usually present in the head and neck or in the upper extremities, but may appear in the posterior mediastinum and more rarely in the retroperitoneum. The latter are often found incidentally or may present with vague, non-specific symptoms if the tumor is large enough to compress surrounding structures.

We report a case of a benign retroperitoneal schwannoma with an unusual clinical presentation, its radiological, histopathological and genetic features, and its subsequent management.

CASE REPORT

A 45-year-old woman was admitted to our emergency department for severe colicky abdominal pain with nausea and vomiting that started about 15 min after her last meal. She had no relevant medical history but mentioned having had back pain for the last 2 mo, which she thought was due to her increasing workload and stress at work. She denied having any abdominal problems until that day. On clinical examination, the patient was afebrile, pale and uncomfortable. Blood pressure and heart rate were normal. Abdominal examination showed a diffusely tender abdomen, more pronounced in the left lower quadrant. She had no guarding or rebound. Deep palpation of the abdomen revealed a non-tender, non-pulsatile mass left of the umbilicus. Rectal examination was unremarkable, except for the presence of a little blood. Within the first hour after admission, the patient suffered one episode of diarrhea stained with a moderate amount of fresh blood. Laboratory tests showed an increased white blood cell count

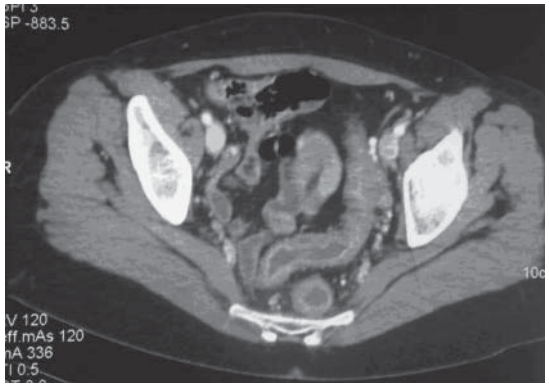

Figure 1 Contrast-enhanced CT scan of the abdomen showing a diffuse infiltration around the rectum and the sigmoid colon, and thickening of their walls.

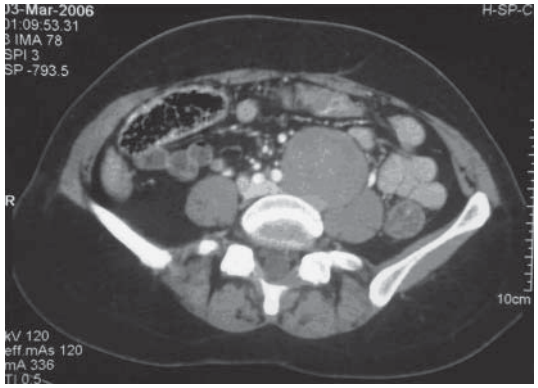

Figure 2 Well-demarcated, homogeneous mass measuring 60×50 mm in close proximity to the left iliac artery, lumbar vertebrae and psoas muscle, on contrast-enhanced CT scanning.

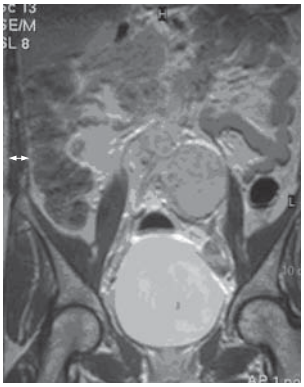

Figure 3 Coronal T_1-weighted MR image using gadolinium, showing a solid mass with the same features as seen with CT scanning.

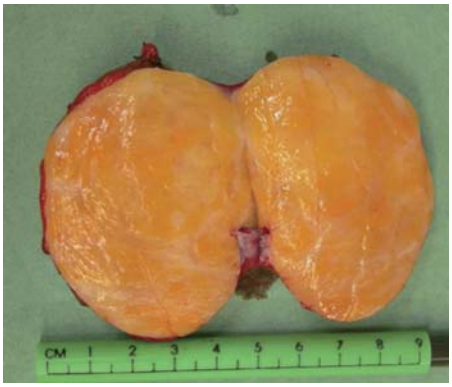

Figure 4 Perioperative examination of the mass revealed a solid, greyish, ovoid tumor with a smooth capsule and a homogeneous yellow core.

(WBC) of 18 000 cells/mm³ and C-reactive protein (CRP) of 1.1 mg/dL. Other values were within the normal range. At this point our differential diagnosis included diverticulitis and a contrast-enhanced computed tomodensitometry (CT) of the abdomen was performed. It revealed a diffuse infiltration around the rectum and the sigmoid colon, and a thickening of their walls (Figure 1). No diverticula were found. It also showed a well-demarcated, homogeneous, left para-aortic mass lying between the lumbar vertebrae and the left psoas muscle, which measured 60 × 50 mm (Figure 2). With few specific findings, the scan was considered inconclusive.

She was hospitalized and received isotonic fluid resuscitation, and intravenous broad-spectrum antibiotics were commenced. Symptoms improved rapidly and disappeared almost completely 3 d after the initial complaints. During her hospital stay, several examinations were performed. First, the patient underwent colonoscopy to exclude inflammatory bowel disease (IBD). It revealed a diffusely edematous, slightly erythematous rectum and sigmoid colon, but no erosive or ulcerative lesions. Histopathological examination of biopsies taken during colonoscopy showed diffuse edema and signs of chronic inflammation, characterized by the presence of mostly lymphocytes and polynuclear granulocytes. Granulomas were absent. A few cryptic abscesses and zones of erosion were also found. The pathologist's diagnosis was chronic colitis, more pronounced in the sigmoid colon than the left colon, and excluded IBD. In laboratory tests, WBC had fallen to a normal value at 9780 cells/mm³ after a rise to 12 000. CRP level followed the same course to settle at 1.7 mg/dL, after it had risen to 10.8. Tumor markers CEA and CA 19.9 were within the normal range. Magnetic resonance imaging showed the same features as seen with CT scanning (Figure 3).

Since we believed that the mass caused the patient's signs and symptoms, we opted for open surgical excision. We approached the retroperitoneal space through a median laparotomy. During exploration and meticulous dissection of the mass, we noticed that it was in close proximity to the aorta and left iliac artery and vein, and seemed to arise from the left para-aortic sympathetic chain. It also adhered partially to the left psoas muscle and to the anterior aspect of the lumbar vertebrae. Complete excision was performed. Perioperative examination of the mass revealed a solid, greyish, spherical tumor with a smooth capsule and a heterogeneous core (Figure 4).

The postoperative course was uneventful. We noticed that the left leg was slightly warmer and dryer than the right, which suggested that we had performed a left lumbar sympathectomy, thus in favor of the excision of a tumor of neural origin. Microscopic histopathological examination revealed strings of spindle cells surrounded by a collagenous stroma that was partially hyalinized and showed cystic degeneration in some regions (Figure 5). There was some nuclear atypia with very limited mitotic activity, but

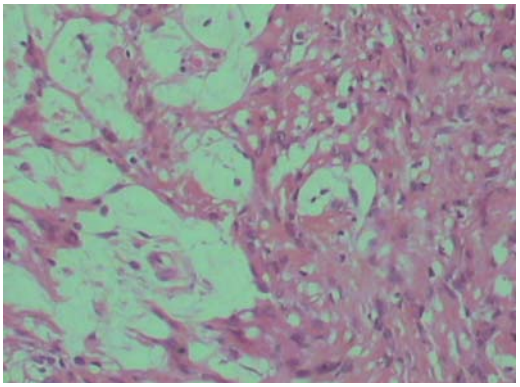

Figure 5 Antoni A area on the right (well-organised spindle cells in a palisade pattern) and Antoni B area (less cellular, loose pleomorphic cells) on the left (HE, × 200).

no signs of malignancy. The tumor tested intensely positive for S-100 protein, which confirmed the diagnosis of benign schwannoma. The integrity of the capsule was also noted, which confirmed total excision of the mass. The patient was discharged from the hospital on the fifth postoperative day.

Six weeks after surgery, she was still symptom free and signs of sympathectomy persisted. A CT scan and colonoscopy were performed to evaluate the evolution of colitis, which had completely resolved. Further investigations were performed to exclude neurofibromatosis type Ⅱ (NF2) or schwannomatosis. Thorough physical examination was performed on the patient but no superficial tumors were found. Family history for schwannoma or meningioma was negative. MRI did not show any tumor of the central nervous system. Furthermore, the presence of a germline *NF2* mutation was excluded by thorough mutation analysis on DNA extracted from the patient' s lymphocytes. We performed direct sequencing of all coding exons of the *NF2* gene. Deletion was excluded by multiplex ligation-dependent probe amplification (MLPA). We concluded that the schwannoma was most likely due to a somatic mutation in the *NF2* gene.

DISCUSSION

Schwannomas, or neurilemmomas, are tumors arising from the Schwann cells of peripheral nerves[1,2]. They are usually found in the head and neck or in the upper extremities. Only 1% is found in the retroperitoneum, which accounts for 0.5%-1.2% of all retroperioneal tumors[3,4]. They can be isolated as sporadic lesions or associated with schwannomatosis or NF2. Although they vary in biological and clinical behavior, their presence is in nearly every case due to alterations or absence of the *NF2* gene (located on chromosome 22q12), which codes for *merlin*, a tumor suppressor protein involved in the growth regulation of Schwann cells, but its exact mechanism has not yet been elucidated. Most schwannomas are benign but (although very rarely) malignant degeneration can occur, and is usually associated with NF2[5]. Patients with benign retroperitoneal schwannomas are predominantly in their second to fifth decade, and women are twice as often affected as men[4,6].

On gross appearance, schwannomas are well-demarcated, solid tumors with a smooth surface and have an ovoid or spherical shape[7]. Sometimes, secondary changes such as hemorrhage, cysts and calcification can be present[1]. They are usually solitary and slow-growing tumors[1]. The retroperitoneum is non-restrictive, so that benign tumors are often able to grow to a large size before causing symptoms. These are generally vague and non-specific[7], and range from lumbar pain and neurological symptoms in the lower extremities[8], to renal colicky pain, with or without hematuria, if it involves the urogenital tract[9]. Abdominal complaints can also occur but are mainly vague and poorly localized, with some digestive disturbances[3,10,11]. Our patient's presentation was peculiar, not only because of the abrupt onset of her symptoms, but also because she had colitis. We believe that the tumor was large enough to compromise venous return in the mesocolon, which led to stasis characterized by the infiltration and parietal thickening seen on CT, the edematous and erythematous aspect seen during colonoscopy, and the chronic inflammation with edema seen on histopathological examination. This idea has been strengthened by the fact that those signs disappeared completely after removal of the tumor.

Diagnosis is rarely made preoperatively. The mass seen on the CT scan showed characteristic features of benign schwannoma. It was a well-demarcated, spherical, solitary mass and in a paravertebral position[6]. Contrast enhancement homogeneity was seen because no gross cystic degeneration or calcification had yet occurred. Most authors agree that these features are not diagnostic. Other diagnoses such as paraganglioma, neurofibroma, ganglioneuroma, tumors of mesodermal origin and retroperitoneal malignancies, including malignant fibrous histiocytoma, lymphoma and liposarcoma, should be considered[12,13]. MRI was helpful because it has a better definition, multiplanar capabilities, and the possibility to differentiate the nature of the tumor, such as solid tissue, fibrous tissue, simple or atypical fluid, and blood[14]. It confirmed the presence of a solid, homogeneous mass, and showed its relation to adjacent structures in greater detail[6,15]. No invasive process was revealed and the margins were still regular, convincing us that the mass was benign in nature[16]. Definite diagnosis was made during histopathological examination and immunohistochemistry. Microscopically, the mass showed Antoni A (well-organised spindle cells in a palisade pattern) and B (less cellular, loose pleomorphic cells) areas[1,2], and tested intensely positive for S-100 protein, which is almost exclusively identified within benign nerve sheath tumors[17,18]. CT-guided fine needle aspiration biopsy can be helpful in determining the origin of a mass preoperatively, but is seldom accurate[7]. Since we believed the mass was causing colitis, open surgical excision was performed. Successful laparoscopic removal of retroperitoneal schwannomas has been reported[19], as well as with endoscopic minilaparotomy[20].

Recurrence is rare and probably due to incomplete excision[7,21]. Further investigations were performed to exclude schwannomatosis or NF2. Absence of other schwannomas and lack of family history of schwannoma theoretically excluded both conditions (see diagnostic criteria in Tables 1 and 2). The presence of a germline *NF2* mutation was excluded by a thorough genetic analysis. We performed direct

Table 1 Diagnostic criteria for NF2[22]

Definite NF2
1 Bilateral vestibular schwannomas or
2 Family history of NF2 (first-degree family relative) plus
   a Unilateral vestibular schwannoma at age < 30 yr, or
   b Any two of the following: meningioma, glioma, schwannoma or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract

Presumptive or probable NF2
1 Unilateral vestibular schwannoma at age < 30 yr plus at least one of the following: meningioma, glioma, schwannoma or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract
2 Multiple meningiomas (two or more) plus
   a Unilateral vestibular schwannoma at age < 30 yr, or
   b One of the following: glioma, schwannoma or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract

Table 2 Diagnostic criteria for schwannomatosis[23]

Definite schwannomatosis
1 Two or more pathologically proved schwannomas, plus
2 Lack of radiographic evidence of vestibular schwannoma at age > 18 yr

Presumptive or probable schwannomatosis
1 Two or more pathologically proved schwannomas without symptoms of eighth nerve dysfunction at age > 30 yr or
2 Two or more pathologically proved schwannomas in an anatomically limited distribution (single limb or segment of the spine), without symptoms of eighth nerve dysfunction, at any age

sequencing of all coding exons of the *NF2* gene. Deletion was excluded by MLPA. This mutation detection strategy, ideally performed on the original tumor specimen, allows the detection of a germline mutation in > 90% of NF2 patients with a family history of the disease, and in > 70% of sporadic cases. However, it is probably only justified in sporadic unilateral vestibular schwannoma in patients aged < 20 years, unless other features of NF2 are present[24].

In conclusion, benign retroperitoneal schwannomas are rare tumors arising from the neural sheath of peripheral nerves. They are usually incidental findings but may become symptomatic if sufficiently large. Symptoms are usually vague and non-specific and can mimic different diseases. CT and MR findings show characteristic features, but none are specific. Diagnosis is based on histopathological examination and immunohistochemistry. Total excision is therapeutic and has a good prognosis. Genetic testing for NF2 is probably not justified in the presence of a solitary retroperitoneal schwannoma.

REFERENCES

1 **Enzinger FM**, Weiss SW. Benign tumors of peripheral nerves. In: Enzinger FM, Weiss SW, ediitors. Soft tissue tumors. 3rd ed. St. Louis: Mosby, 1995: 821-888
2 **Kyriakos ML**. Tumors and tumorlike conditions of soft tissues. In: Kissane JM, editor. Anderson's pathology. 8th ed. St. Louis: Mosby, 1985: 1682-1687
3 **McCarthy S**, Duray PH. Giant retroperitoneal neurilemoma: a rare cause of digestive tract symptoms. *J Clin Gastroenterol* 1983; **5**: 343-347
4 **Scanlan DB**. Primary retroperitoneal tumors. *J Urol* 1959; **81**: 740-745
5 **Evans DG**, Sainio M, Baser ME. Neurofibromatosis type 2. *J Med Genet* 2000; **37**: 897-904
6 **Kim SH**, Choi BI, Han MC, Kim YI. Retroperitoneal neurilemoma: CT and MR findings. *AJR Am J Roentgenol* 1992; **159**: 1023-1026
7 **Daneshmand S**, Youssefzadeh D, Chamie K, Boswell W, Wu N, Stein JP, Boyd S, Skinner DG. Benign retroperitoneal schwannoma: a case series and review of the literature. *Urology* 2003; **62**: 993-997
8 **Schindler OS**, Dixon JH, Case P. Retroperitoneal giant schwannomas: report on two cases and review of the literature. *J Orthop Surg* (Hong Kong) 2002; **10**: 77-84
9 **Singh V**, Kapoor R. Atypical presentations of benign retroperitoneal schwannoma: report of three cases with review of literature. *Int Urol Nephrol* 2005; **37**: 547-549
10 **Hurley L**, Smith JJ 3rd, Larsen CR, Silverman ML. Multiple retroperitoneal schwannomas: case report and review of the literature. *J Urol* 1994; **151**: 413-416
11 **Miller PL**, Tessler A, Alexander S, Pinck BD. Retroperitoneal neurilemmoma. *Urology* 1978; **11**: 619-623
12 **Kransdorf MJ**. Benign soft-tissue tumors in a large referral population: distribution of specific diagnoses by age, sex, and location. *AJR Am J Roentgenol* 1995; **164**: 395-402
13 **Hughes MJ**, Thomas JM, Fisher C, Moskovic EC. Imaging features of retroperitoneal and pelvic schwannomas. *Clin Radiol* 2005; **60**: 886-893
14 **Hayasaka K**, Tanaka Y, Soeda S, Huppert P, Claussen CD. MR findings in primary retroperitoneal schwannoma. *Acta Radiol* 1999; **40**: 78-82
15 **Cretella JP**, Rafal RB, McCarron JP Jr, Markisz JA. MR imaging in the diagnosis of a retroperitoneal schwannoma. *Comput Med Imaging Graph* 1994; **18**: 209-212
16 **Nakashima J**, Ueno M, Nakamura K, Tachibana M, Baba S, Deguchi N, Tazaki H, Murai M. Differential diagnosis of primary benign and malignant retroperitoneal tumors. *Int J Urol* 1997; **4**: 441-446
17 **Weiss SW**, Langloss JM, Enzinger FM. Value of S-100 protein in the diagnosis of soft tissue tumors with particular reference to benign and malignant Schwann cell tumors. *Lab Invest* 1983; **49**: 299-308
18 **Kawahara E**, Oda Y, Ooi A, Katsuda S, Nakanishi I, Umeda S. Expression of glial fibrillary acidic protein (GFAP) in peripheral nerve sheath tumors. A comparative study of immunoreactivity of GFAP, vimentin, S-100 protein, and neurofilament in 38 schwannomas and 18 neurofibromas. *Am J Surg Pathol* 1988; **12**: 115-120
19 **Ohigashi T**, Nonaka S, Nakanoma T, Ueno M, Deguchi N. Laparoscopic treatment of retroperitoneal benign schwannoma. *Int J Urol* 1999; **6**: 100-103
20 **Kageyama Y**, Kihara K, Ishizaka K, Okuno T, Kawakami S, Fujii Y, Masuda H, Suzuki M, Hyochi N, Arai G, Saito K, Sakai Y. Endoscope-assisted minilaparotomy (endoscopic minilaparotomy) for retroperitoneal Schwannoma: experience with three cases. *Jpn J Clin Oncol* 2002; **32**: 177-180
21 **Carpenter WB**, Kernohan JW. Retroperitoneal ganglioneuromas and neurofibromas. A clinicopathological study. *Cancer* 1963; **16**: 788-797
22 **Gutmann DH**, Aylsworth A, Carey JC, Korf B, Marks J, Pyeritz RE, Rubenstein A, Viskochil D. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA* 1997; **278**: 51-57
23 **Jacoby LB**, Jones D, Davis K, Kronn D, Short MP, Gusella J, MacCollin M. Molecular analysis of the NF2 tumor-suppressor gene in schwannomatosis. *Am J Hum Genet* 1997; **61**: 1293-1302
24 **Evans DG**, Ramsden RT, Gokhale C, Bowers N, Huson SM, Wallace A. Should NF2 mutation screening be undertaken in patients with an apparently isolated vestibular schwannoma? *Clin Genet* 2007; **71**: 354-358

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CASE REPORT

Gallstone spillage caused by spontaneously perforated hemorrhagic cholecystitis

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Abstract

There are occasional incidences of gallstone spillage during laparoscopic cholecystectomy, and there have been frequent reports on such a topic in the literature. To the best of our knowledge, however, there have been no reports about spilled stones caused by spontaneously perforated hemorrhagic cholecystitis. Here, we report the radiologic findings of spilled stones caused by spontaneously perforated hemorrhagic cholecystitis in a 55-year-old man.

Key words: Gallbladder perforation; Gallstone spillage; Hemorrhagic cholecystitis

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INTRODUCTION

With the increased use of laparoscopic surgery, the spillage of gallstones during laparoscopic cholecystectomy has been reported in 6%-40% of cases[1,2]. To the best of our knowledge, however, there have been no reports about spilled stones caused by spontaneously perforated hemorrhagic cholecystitis. Here, we present ultrasonography (US) and computed tomography (CT) images of this rare condition.

CASE REPORT

A 55-year-old man complained of abrupt upper abdominal pain during hospitalization for a brain abscess. A complete blood count taken 12 h after the attack showed that the level of hemoglobin dropped to 8.8 g/L (from 13.3 g/L, 36 h before the attack). Other blood analysis revealed mild thrombocytopenia (platelet count $106 \times 10^3/\mu L$), and mild hyperbilirubinemia (total bilirubin concentration 1.7 mg/dL); however, the white blood cell count was normal ($9.27 \times 10^6/\mu L$).

Immediately after the attack, the patient underwent US, which demonstrated echogenic material in the gallbladder lumen (Figure 1A), with a positive sonographic Murphy's sign. US was discontinued because the patient complained of severe abdominal pain. Contrast-enhanced CT was performed and its images revealed high-density fluid, both inside and outside the gallbladder. One impacted cystic duct stone was seen, as were several calcified objects (which looked like stones), within the high-density (46-61 HU) fluid surrounding the gallbladder (Figure 1B and C). A defect in the wall or mucosal disruption of the gallbladder was also noted (Figure 1D and E). In addition, underlying liver cirrhosis with splenomegaly was observed. Percutaneous transhepatic gallbladder drainage (PTGBD) and cholangiography were performed. Cholecystography demonstrated contrast leakage from the gallbladder (Figure 1F).

He had a medical history of several years of alcoholic liver cirrhosis with mild esophageal varices and multiple gallbladder stones. Two months before the current attack, he underwent an abdominal CT scan to evaluate liver cirrhosis. At that time, CT revealed multiple stones in the gallbladder, without complications (Figure 1G).

DISCUSSION

Laparoscopic cholecystectomy has become a popular alternative to open surgery for the treatment of gallstones. With the increase in laparoscopic cholecystectomy, the incidence of gallstone spillage has increased, with an incidence ranging from 6% to 40%[1,2]. The complications of peritoneal spilled gallstones are abscess, fistula formation within various intraperitoneal organs, or sinus tract formation[3-7]. To the best of our knowledge, however, gallstone spillage caused by spontaneously perforated hemorrhagic cholecystitis in a patient who did not undergo cholecystectomy has not been reported.

The proposed mechanism of gallbladder perforation is stone impaction in the cystic duct, which leads to retention of secretion from mucus glands and distention, with progressive distention leading to vascular compromise, followed by necrosis and perforation[8]. During this process, bleeding can occur, which results in hemorrhagic chole-

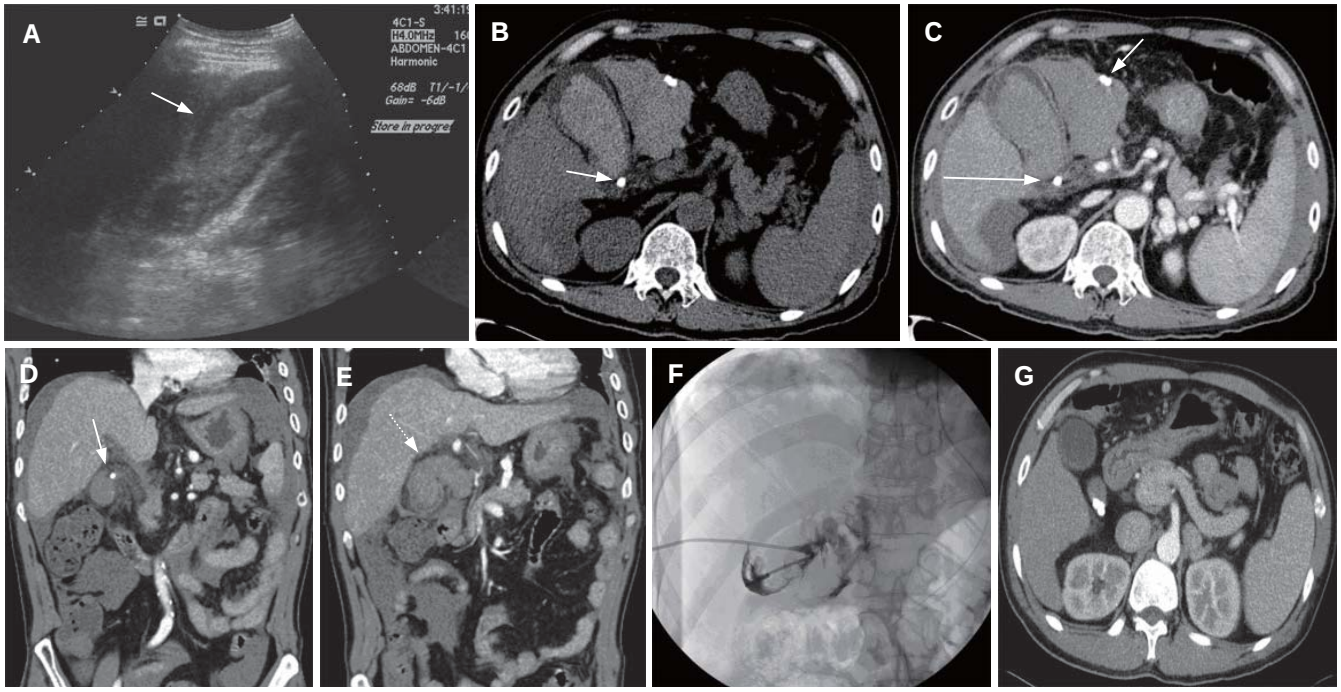

Figure 1 A 55-year-old man with right upper quadrant pain. US images (**A**) demonstrate heterogeneous, highly echogenic material, both within and outside the gallbladder lumen (arrows), with a positive sonographic Murphy's sign. Non-contrast (**B**) and contrast-enhanced (**C**) transverse CT images show high-attenuation (46-61HU) material, both in the gallbladder lumen and pericholecystic space. One stone is seen in the cystic duct (long arrow) and calcified material (with the same appearance as the cystic duct stone) is seen in the fluid collected (short arrow) around the gallbladder. Contrast-enhanced coronal CT images (**D**, **E**) show well the impacted cystic-duct stone (arrow), and the mucosal defect with continuation of hemorrhage (dotted arrow). PTGBD (**F**) with cholecystography demonstrates contrast leakage from the gallbladder. Contrast-enhanced transverse CT images (**G**) taken 2 mo before the current attack show multiple stones in the gallbladder neck without complications.

cystitis with hemoperitoneum. In our case, the patient had liver cirrhosis and therefore the risk of bleeding could have been increased.

In our study, US showed heterogeneous, highly echogenic material, both within and outside the gallbladder lumen, which may have been suggestive of gallbladder perforation with hemorrhage. However, we could not detect the exact perforation site nor spilled gallstones on US, and so we were not able to diagnose gallbladder perforation at that time. CT clearly demonstrated the perforation site at the gallbladder wall and spilled radiopaque stones that were missed on US. It has been reported that distended gallbladder, thickened and bulging gallbladder wall, pericholecystic fluid, cholelethiasis, and gallbladder wall defects are the US and CT findings of gallbladder perforation. The most specific of these findings is gallbladder wall defects, with a detection rate of 38.4% on US and 69.2% on CT[9]. The most common site of perforation is reported to be the fundus (70% of cases), due to poor vascular supply[10].

In conclusion, gallstone spillage due to spontaneous perforation is a very rare condition. However, US or CT visualization of mucosal disruption of the gallbladder wall, with gallstones within hemoperitoneum is suggestive of the condition.

REFERENCES

1 **Fletcher DR**, Hobbs MS, Tan P, Valinsky LJ, Hockey RL, Pikora TJ, Knuiman MW, Sheiner HJ, Edis A. Complications of cholecystectomy: risks of the laparoscopic approach and protective effects of operative cholangiography: a population-based study. *Ann Surg* 1999; **229**: 449-457

2 **Ahmad SA**, Schuricht AL, Azurin DJ, Arroyo LR, Paskin DL, Bar AH, Kirkland ML. Complications of laparoscopic cholecystectomy: the experience of a university-affiliated teaching hospital. *J Laparoendosc Adv Surg Tech A* 1997; **7**: 29-35

3 **Woodfield JC**, Rodgers M, Windsor JA. Peritoneal gallstones following laparoscopic cholecystectomy: incidence, complications, and management. *Surg Endosc* 2004; **18**: 1200-1207

4 **Diez J**, Arozamena C, Gutierrez L, Bracco J, Mon A, Sanchez Almeyra R, Secchi M. Lost stones during laparoscopic cholecystectomy. *HPB Surg* 1998; **11**: 105-108; discuss 108-109

5 **Hui TT**, Giurgiu DI, Margulies DR, Takagi S, Iida A, Phillips EH. Iatrogenic gallbladder perforation during laparoscopic cholecystectomy: etiology and sequelae. *Am Surg* 1999; **65**: 944-948

6 **Rice DC**, Memon MA, Jamison RL, Agnessi T, Ilstrup D, Bannon MB, Farnell MB, Grant CS, Sarr MG, Thompson GB, van Heerden JA, Zietlow SP, Donohue JH. Long-term consequences of intraoperative spillage of bile and gallstones during laparoscopic cholecystectomy. *J Gastrointest Surg* 1997; **1**: 85-90; discussion 90-91

7 **Schafer M**, Suter C, Klaiber C, Wehrli H, Frei E, Krahenbuhl L. Spilled gallstones after laparoscopic cholecystectomy. A relevant problem? A retrospective analysis of 10,174 laparoscopic cholecystectomies. *Surg Endosc* 1998; **12**: 305-309

8 **Madrazo BL**, Francis I, Hricak H, Sandler MA, Hudak S, Gitschlag K. Sonographic findings in perforation of the gallbladder. *AJR Am J Roentgenol* 1982; **139**: 491-496

9 **Kim PN**, Lee KS, Kim IY, Bae WK, Lee BH. Gallbladder perforation: comparison of US findings with CT. *Abdom Imaging* 1994; **19**: 239-242

10 **Massie JR Jr**, Coxe JW 3rd, Parker C, Dietrick R. Gall bladder perforations in acute cholecystitis. *Ann Surg* 1957; **145**: 825-831

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CASE REPORT

Mirizzi syndrome in an anomalous cystic duct: A case report

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Abstract

Mirizzi syndrome is a rare complication of gallstone disease, and results in partial obstruction of the common bile duct or a cholecystobiliary fistula. Moreover, congenital anatomical variants of the cystic duct are common, occurring in 18%-23% of cases, but Mirizzi syndrome underlying an anomalous cystic duct is an important clinical consideration. Here, we present an unusual case of type I Mirizzi syndrome with an uncommon anomalous cystic duct, namely, a low lateral insertion of the cystic duct with a common sheath of cystic duct and common bile duct.

Key words: Bile duct diseases and surgery; Cholelithiasis; Cholelithiasis and surgery; Cystic duct; Cholangiography

Jung CW, Min BW, Song TJ, Son GS, Lee HS, Kim SJ, Um JW. Mirizzi syndrome in an anomalous cystic duct: A case report. *World J Gastroenterol* 2007; 13(41): 5527-5529

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INTRODUCTION

Mirizzi described a functional hepatic syndrome in 1948, which consists of obstruction of the common hepatic duct secondary to compression by an impacted gallstone in the cystic duct or in the infundibula of the gallbladder (GB) associated with an inflammatory response involving the cystic duct and the common bile duct (CBD), surrounding inflammation, recurrent cholangitis, and spasm of the circular muscular sphincter in the hepatic duct[1]. Mirizzi syndrome (MS) indicates a narrowing of the common bile duct by a gallstone impacted in the cystic duct or a cholecystobiliary fistula. Many cases have so far been reported, and various operations have been suggested depending on the types of MS. Laparoscopic cholecystectomy has become the standard operation for gallstones, and many authors have adopted this operation for type I MS. However, high rates of conversion and bile duct injury indicate that this is not a safe treatment modality for MS, especially when combined with a cystic duct anomaly[2-5]. In this report, we present a case of type I Mirizzi syndrome, complicated by a rare anomalous cystic duct, which was operated with an open procedure.

CASE REPORT

A 34-year-old Korean female presented at our hospital complaining of abdominal pain of 2-d duration. The finding of her physical examination was not remarkable except for tenderness at the right upper abdominal quadrant with a positive Murphy sign. Furthermore, her laboratory findings were not remarkable except for an abnormal liver function: 2.1/1.6 mg/dL bilirubin (total/direct), 126 IU/L alkaline phosphatase, 310 IU/L aspartate aminotransferase, 625 IU/L alanine aminotransferase, and 114 IU/L gamma glutamyl transpeptidase. Ultrasonography (US) revealed multiple gallstones with GB wall thickening and a suspicious 1 cm-sized distal CBD stone. The same findings were also noted on endoscopic ultrasonography (EUS). Endoscopic retrograde cholangiopancreatography (ERCP) was conducted for a closer examination and removal of the CBD stone. However, ERCP showed that the 1 cm-sized gallstone was impacted in an anomalous cystic duct joined with the distal CBD. In addition, a gallstone compressed the CBD at the level of union between the pancreatic and biliary ducts (Figure 1). Mirizzi syndrome with low medial insertion of the cystic duct was preoperatively diagnosed, and endoscopic nasobiliary drainage (ENBD) tube was placed to decompress the biliary obstruction after endoscopic sphincterotomy.

During laparotomy via a right subcostal incision, the GB was found to be shrunken, thickened, and inflamed. The long and dilated cystic duct seen on ERCP was not identified in the operative field, and the GB was strongly attached to the CBD because of chronic inflammation and also possible anomalous union to the CBD (i.e., possibly due to a common sheath cystic duct and CBD). After subtotal cholecystectomy, the remnant of cystic duct was thickened and dilated to about 1.5 cm in diameter. Intraoperative choledochoscopy was performed *via* the

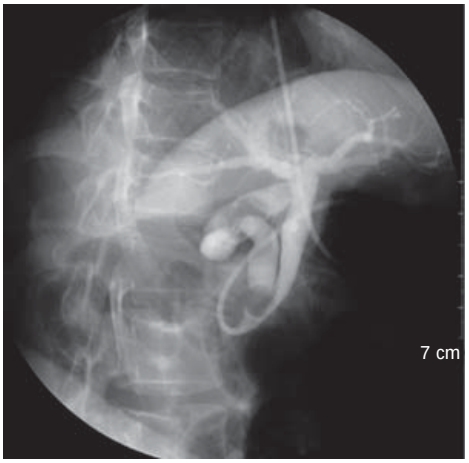

Figure 1 ENBD cholangiogam after endoscopic retrograde cholangiography showing a 1 cm-sized gall stone impacted in a cystic duct joining the extreme lower CBD portion and other stones in gall bladder. Mild CBD dilation was noted due to the presence of a cystic duct stone.

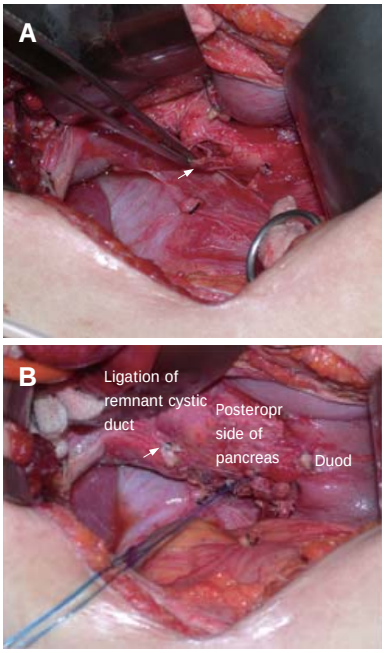

Figure 2 Torn pancreas and intrapancreatic portion of the cystic duct (**A**) and their primary repair (**B**) during lithotripsy of the impacted stone.

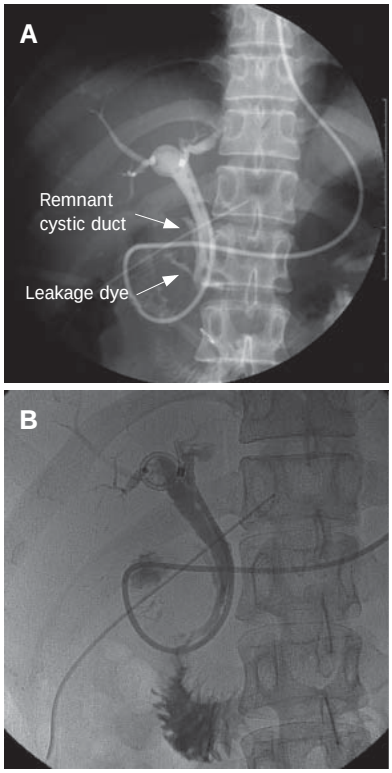

Figure 3 Follow-up ENBD cholangiogram showing minor leakage from the repaired cystic duct on the 7th postoperative day (**A**), but no visible leakage on the 21st postoperative day (**B**).

remnant of cystic duct and the impacted cystic duct stone was visualized. However, lithotripsy with choledochoscopy failed at this time. Another attempt was made to remove the cystic duct stone after duodenum mobilization through the remnant of cystic duct using a stone forceps made. Moreover, during this procedure, the pancreas and intrapancreatic portion of the cystic duct were torn, and the impacted cholesterol stone escaped retroperitoneally from the lacerated intrapancreatic cystic duct and pancreas (Figure 2A). After ligation of the stump of remaining cystic duct and primary repair of the torn cystic duct and pancreas (Figure 2B), intraoperative cholangiography *via* an ENBD tube showed neither leakage nor residual stone. A closed suction type drain was placed in the liver bed and retroduodenal space, and the abdominal wall was then closed. Because the ENBD catheter and endoscopic sphincterotomy were preoperatively performed, they could have played a role in biliary drainage in place of choledochotomy and T-tube insertion. The postoperative course was uneventful, except for minimal drainage from the closed suction drain and minor leakage from the repaired cystic duct, which was visible on the 7th postoperative day on ENBD cholangiography (Figure 3A). After conservative management, no visible leakage was observed from the repaired cystic duct on follow-up ENBD cholangiography on the 21st postoperative day (Figure 3B). After removal of the ENBD catheter, the patient was discharged on the postoperative 23rd d, and has now been doing well for over 3-years.

DISCUSSION

Congenital anatomical variants of the cystic duct are common, occurring in 18%-23% of cases. Among those cases, the cystic duct inserts into the middle third of the extrahepatic bile duct in 75% of cases and into the distal third in 10% of cases. Five types of cystic duct anomaly have been described: a long cystic duct with low fusion with the CHD, abnormally high fusion between a cystic duct and the CHD, accessory hepatic duct, cystic duct entering the right hepatic duct, and cholecystohepatic duct[6-8]. In particular, low medial insertion of the cystic duct deserves special attention, because this anatomical variant may lead to misdiagnosis by imaging studies, thus adversely affecting therapeutic intervention. In addition, anomalous cystic duct may also be a problem at cholecystectomy[9]. In the presently described case, ERCP demonstrated that the biliary obstruction was caused by a cystic duct gallstone in a low medially inserting cystic duct which joined the distal CBD near the ampulla of Vater, but not by a distal CBD stone as indicated by US and EUS.

Zhou[10] reported that 65 (5.9%) among 1100 cases had an abnormal cystic duct and common bile duct

confluence, as determined by ERCP, and he divided cystic duct anomalies into three types, and the very low-sited confluence was found in 9 (13.8%) cases among anomalous cystic ducts. Another study of 50 patients reported a single case (2%) of intrapancreatic confluence[11].

MS is a rare disease entity that accounts for about 0.1%-2.5% of all operations performed for gall bladder stones[12,13]. In 1982, McSherry *et al*[14] classified MS into two types, based on ERCP findings. Type I involves external compression of the common hepatic duct by a large stone impacted in the cystic duct, or the Hartmann's pouch, without any lesion in the gall bladder or common hepatic duct wall. In type Ⅱ, a cholecystocholedochal fistula is present, which is caused by a calculus that has eroded partly or completely into the common duct. In 1989, Csendes *et al*[12] classified MS into four types, and their classification categorized cholecystocholedochal fistula further depending on its extent of destruction. The McSherry classification or the Csendes classification has usually been used by clinicians, because these classifications more usefully guide surgical management.

MS has been highlighted because of its high incidence of iatrogenic biliary injuries and its demand for complex surgical procedures. Preoperative diagnosis of MS is difficult. However, it is important to prevent unexpected intraoperative morbidities, such as bile duct injury.

In the present case, a cystic duct stone was misdiagnosed as a CBD stone by US and EUS. However, in order to evaluate and remove the stone, ERCP was carried out and finally, a cystic duct stone with MS type I combined with a low lying anomalous cystic duct was diagnosed before surgery. Most MS cases have CBD obstruction symptoms such as jaundice (76.5%), which induce surgeons to attend to CBD problems[2]. However, cases not associated with CBD obstruction symptoms may be diagnosed as GB stone requiring only laparoscopic cholecystectomy. In a series reported by Tan *et al*[5], bile duct injuries were observed in 4 (16.7%) of 24 operatively treated patients, and all the 4 injuries occurred in patients without a preoperative diagnosis.

Surgical treatment of MS depends on its type. Although laparoscopic cholecystectomy has almost completely replaced open cholecystectomy for the treatment of symptomatic gallstone disease, laparoscopic cholecystectomy is relatively hazardous in patients with MS, because safe dissection of Calot's triangle is difficult due to severe local inflammations and adhesions[4]. Al-Akeely *et al*[2] reported that 2 of 6 type I MS patients successfully underwent laparoscopic partial cholecystectomy with an endo-GIA stapler. However, the procedure was converted to an open procedure in the remaining 4 patients. Schafer *et al*[4] reported that conversion to an open approach was needed in 24 of 34 patients (74%) with type I MS and in all 5 patients with type Ⅱ MS.

In the present case, open cholecystectomy was performed for MS combined with a cystic duct anomaly. However, bile duct injury occurred during removal of the impacted cystic duct stone. Thus, we would like to advise that, if MS combined with a cystic duct anomaly is diagnosed before surgery, the operator should not hesitate to perform open surgery and dissect carefully, while considering anatomical deformities associated with chronic inflammation. Moreover, intraoperative cholangiography should be performed to minimize the risk of biliary injury.

REFERENCES

1 **Mirizzi PL**. Sindrome del conducto hepatico. *J Int Chirur* 1948; **1**: 219-225
2 **Al-Akeely MH**, Alam MK, Bismar HA, Khalid K, Al-Teimi I, Al-Dossary NF. Mirizzi syndrome: ten years experience from a teaching hospital in Riyadh. *World J Surg* 2005; **29**: 1687-1692
3 **Waisberg J**, Andre EA, Franco MI, Abucham-Neto JZ, Wickbold D, Goffi FS. Curative resection plus adjuvant chemotherapy for early stage primary gastric non-Hodgkin's lymphoma: a retrospective study with emphasis on prognostic factors and treatment outcome. *Arq Gastroenterol* 2006; **43**: 30-36
4 **Schafer M**, Schneiter R, Krahenbuhl L. Incidence and management of Mirizzi syndrome during laparoscopic cholecystectomy. *Surg Endosc* 2003; **17**: 1186-1190; discussion 1191-1192
5 **Tan KY**, Chng HC, Chen CY, Tan SM, Poh BK, Hoe MN. Mirizzi syndrome: noteworthy aspects of a retrospective study in one centre. *ANZ J Surg* 2004; **74**: 833-837
6 **Benson EA**, Page RE. A practical reappraisal of the anatomy of the extrahepatic bile ducts and arteries. *Br J Surg* 1976; **63**: 853-860
7 **Puente SG**, Bannura GC. Radiological anatomy of the biliary tract: variations and congenital abnormalities. *World J Surg* 1983; **7**: 271-276
8 **Shaw MJ**, Dorsher PJ, Vennes JA. Cystic duct anatomy: an endoscopic perspective. *Am J Gastroenterol* 1993; **88**: 2102-2106
9 **Ghahremani GG**. Postsurgical biliary tract complications. *Gastroenterologist* 1997; **5**: 46-57
10 **Zhou YB**. Cystic duct anomalies found by ERCP and their clinical implications. *Zhonghua Waike Zazhi* 1990; **28**: 328-330, 380
11 **Ichii H**, Takada M, Kashiwagi R, Sakane M, Tabata F, Ku Y, Fujimori T, Kuroda Y. Three-dimensional reconstruction of biliary tract using spiral computed tomography for laparoscopic cholecystectomy. *World J Surg* 2002; **26**: 608-611
12 **Csendes A**, Diaz JC, Burdiles P, Maluenda F, Nava O. Mirizzi syndrome and cholecystobiliary fistula: a unifying classification. *Br J Surg* 1989; **76**: 1139-1143
13 **Shah OJ**, Dar MA, Wani MA, Wani NA. Management of Mirizzi syndrome: a new surgical approach. *ANZ J Surg* 2001; **71**: 423-427
14 **McSherry CK**, Ferstenberg H, Virshup M. The Mirizzi syndrome: suggested classification and surgical therapy. *Surg Gastroenterol*. 1982; **1**: 219-225

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CASE REPORT

Hepatic abscess secondary to a rosemary twig migrating from the stomach into the liver

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Abstract

The ingestion of a foreign body that penetrates the gastric wall and migrates to the liver, where it causes an abscess is uncommon. A case of an ingested rosemary twig perforating the gastric antrum, then migrating to the liver, complicated by hepatic abscess and Staphylococcus aureus sepsis is reported. A 59-year-old man without a history of foreign body ingestion was admitted to our hospital because of sepsis and epigastralgia, which had progressively worsened. No foreign body was identified at preoperative imaging, but a rosemary twig was discovered during laparotomy. The liver abscess and sepsis were controlled successfully with surgery and antibiotics. This unusual condition should be kept in mind when dealing with cases of hepatic abscess, or even sepsis of unknown origin. Despite the improvement of non-surgical techniques such as percutaneous drainage and interventional endoscopy, surgery still remains important in the treatment of hepatic abscess caused by an ingested foreign body.

Key words: Foreign Body; Gastrointestinal perforation; Hepatic Abscess; Ingestion; Migration

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INTRODUCTION

The first case of hepatic abscess as a result of gastrointestinal tract (GIT) perforation caused by a foreign body was published by Lambert in 1898[1]. Most foreign bodies pass through the GIT without causing any damage once they pass the lower esophageal sphincter[2]. It is not unusual to come across patients in clinical practice with GIT perforation due to ingested foreign bodies, but the development of a secondary hepatic abscess due to foreign body perforation of the gastric wall is a rare condition[1-4]. In the majority of cases, an early diagnosis is difficult to make because of the non-specific clinical presentation[3].

CASE REPORT

A 59-year-old man presented with epigastric, right upper abdominal pain and intermittent high-grade fever, with chills and rigors for the past 2 mo. There was no history of foreign body ingestion. He had received treatment for his fever of unknown origin at a district hospital in the form of antibiotics (ceftriaxone, 2.0 g daily) and antipyretics. On admission, examination revealed a septic, high-grade febrile patient (38.9℃) with tachycardia (pulse 128 bpm) and tachypnoea (21 breaths/min). The white blood cell count was 28.8 × 10^9/L, alkaline phosphatase was 180 U/L, bilirubin was normal, and C-reactive protein (CRP) was 320 mg/L. The abdomen was tense with tenderness in the epigastrium and right hypochondrium, without any signs of peritoneal reaction. Chest X-ray revealed a right-sided pleural effusion, but X-ray of the abdomen was normal. Ultrasound (US) examination, contrast-enhanced computerized tomography (CT) (Figure 1) and magnetic resonance imaging (MRI) of the abdomen (Figure 2) revealed a hepatic abscess of 11.7 cm × 10.3 cm × 8.8 cm in the segments S4b-S5. The patient was started on meropenem and subjected to exploratory laparotomy, which revealed a huge abscess occupying the central segments of the liver, and concomitant acute calculous cholecystitis. There was no association between the inflammatory process in the gallbladder and abscess formation. After cholecystectomy, hepatotomy along the gallbladder bed was performed, and about 500 mL of pus

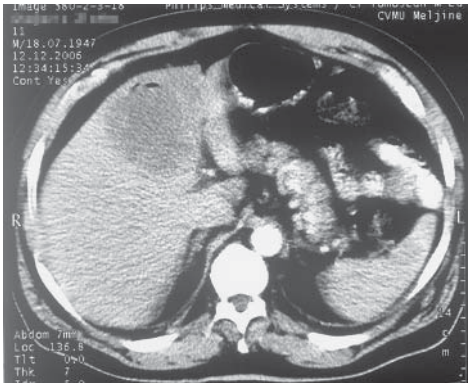

Figure 1 CT scan of the abdomen demonstrating a liver abscess containing a small amount of gas.

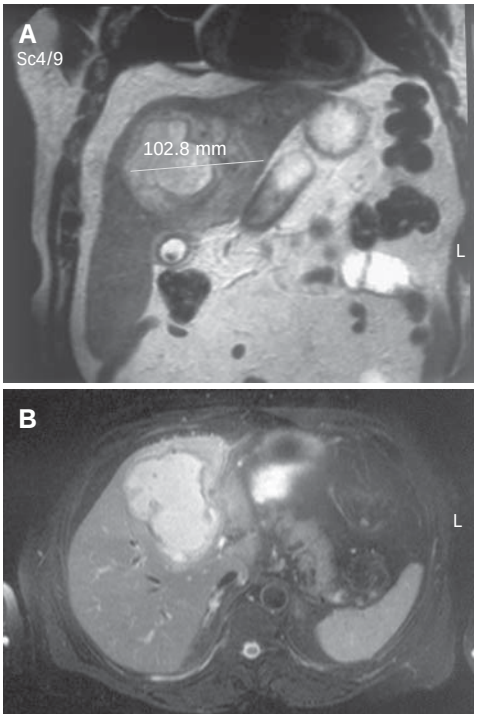

Figure 2 MRI of the abdomen showing the abscess in liver segments S4b-S5. (**A**) T2W coronal view; (**B**) T2W axial view.

was drained and a rosemary twig of 4.5 cm was retrieved from the abscess cavity (Figure 3). Since there was no obvious fistulous communication between the liver and stomach or duodenum, a careful examination of the upper GIT revealed a small covered perforation of the gastric antrum wall. The perforation was repaired by using single-layer interrupted sutures (Figure 4). The abscess was also drained with a triple-tube lavage system. Vancomycin was added postoperatively due to subsequent blood culture that showed Staphylococcus aureus. The patient recovered uneventfully and was discharged on postoperative d 10.

DISCUSSION

The reported incidence of foreign bodies penetrating the GIT is < 1%[1,2,5], with the objects being pointed or sharp in most cases, such as sewing needles, tooth picks, and

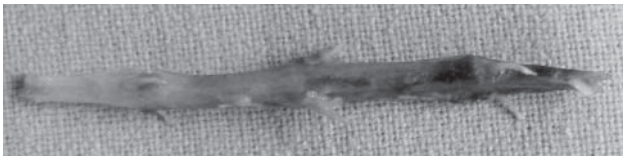

Figure 3 Rosemary twig after retrieval from the hepatic abscess.

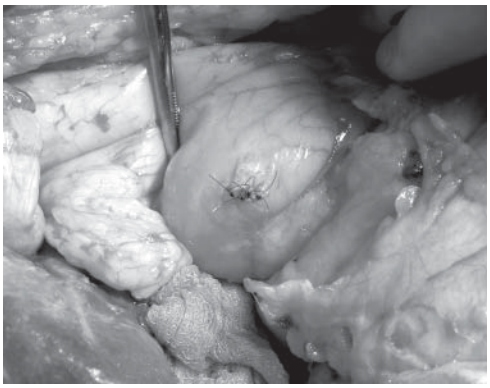

Figure 4 Repair of the perforation site in the gastric antrum.

chicken and fish bones[5-8], pens, toothbrushes and dental plates[9-11].

The most common sites of perforation of the GIT are the stomach and duodenum[1-3]. Abscess formation occurs more often on the left hemiliver[2,3]. The ingestion of a foreign body that penetrates the GIT wall and migrates to the liver, where it causes an abscess is indeed rare, with 46 cases being reported in the recent literature[3]. No report of hepatic abscess caused by ingestion of a rosemary twig (used for food flavoring), has been found so far in the medical literature.

The classical presenting features of hepatic abscess, such as fever with chills, abdominal pain and discomfort, and jaundice are present in only a small number of patients[1-4]. Most patients have non-specific symptoms such as anorexia, vomiting or weight loss with leucocytosis[6-9], or increased transaminases, bilirubin or alkaline phosphatase[10,11]. The migrating foreign body may remain silent for a long time and may only be discovered if there are features of infection or abscess formation[1]. The presentation of a penetrating foreign body (tooth pick) as a granulomatous liver abscess has been reported 1 mo after ingestion, requiring partial lateral resection of the liver[8]. The prolonged time course of the illness, the lack of history of foreign body ingestion, the relatively non-specific symptoms and signs, and the non-specific results obtained by using conventional radiography have resulted in delayed recognition of this possibly fatal diseas [4]. Deaths caused by missed or delayed diagnosis have been reported, one of which was discovered on autopsy[12-14]. Thus, both a high clinical suspicion index and prompt treatment are necessary for this rare condition[1-3,11-15]. An ingested foreign body may be identified with plain X-rays of the abdomen, if the body is radio-opaque. Other methods of foreign body identification include US, CT, MRI, upper GIT endoscopy, colonoscopy,

and laparotomy[8,15-16]. Endoscopy may be helpful when performed early, before the foreign body migration and mucosal healing[3,7].

The recommended treatment is exploratory laparotomy to evacuate the hepatic abscess, remove the foreign body, and repair the perforation site in the GIT[1-3,16]. Since the gastric perforation is small and is probably covered by the omentum or hepatic lobe, minimally invasive treatment such as percutaneous drainage of the pus collection, combined with endoscopic removal of the foreign body, can be employed to reduce open surgery[2,9]. Also, the successful treatment of hepatic abscess and foreign body removal by the percutaneous transhepatic approach has been reported[10].

In conclusion, we report a very rare case of the migration of an ingested rosemary twig into the liver through the stomach, which resulted in hepatic abscess and sepsis. Due to the lack of obvious fistulous communication between the liver and upper GIT, careful exploration of the abscess cavity is of great importance. This condition should be kept in mind when dealing with cases of hepatic abscess, or even sepsis of unknown origin. Therefore, an early diagnosis and prompt intervention are optimal for treatment and necessary to avoid death. Despite the improvement of non-surgical techniques such as percutaneous drainage and interventional endoscopy, surgery still remains important in the treatment of hepatic abscess caused by an ingested foreign body.

REFERENCES

1 **Chintamani C**, Singhal V, Lubhana P, Durkhere R, Bhandari S. Liver abscess secondary to a broken needle migration--a case report. *BMC Surg* 2003; **3**: 8

2 **Lee KF**, Chu W, Wong SW, Lai PB. Hepatic abscess secondary to foreign body perforation of the stomach. *Asian J Surg* 2005; **28**: 297-300

3 **Santos SA**, Alberto SC, Cruz E, Pires E, Figueira T, Coimbra E, Estevez J, Oliveira M, Novais L, Deus JR. Hepatic abscess induced by foreign body: case report and literature review. *World J Gastroenterol* 2007; **13**: 1466-1470

4 **Starakis I**, Karavias D, Marangos M, Psoni E, Bassaris H. A rooster's revenge: hepatic abscess caused by a chicken bone. *Eur J Emerg Med* 2005; **12**: 41-42

5 **Theodoropoulou A**, Roussomoustakaki M, Michalodimitrakis MN, Kanaki C, Kouroumalis EA. Fatal hepatic abscess caused by a fish bone. *Lancet* 2002; **359**: 977

6 **Kumar S**, Gupta NM. Foreign bodies migrating from gut to liver. *Indian J Gastroenterol* 2000; **19**: 42

7 **Bilimoria KY**, Eagan RK, Rex DK. Colonoscopic identification of a foreign body causing an hepatic abscess. *J Clin Gastroenterol* 2003; **37**: 82-85

8 **Kanazawa S**, Ishigaki K, Miyake T, Ishida A, Tabuchi A, Tanemoto K, Tsunoda T. A granulomatous liver abscess which developed after a toothpick penetrated the gastrointestinal tract: report of a case. *Surg Today* 2003; **33**: 312-314

9 **Chiang TH**, Liu KL, Lee YC, Chiu HM, Lin JT, Wang HP. Sonographic diagnosis of a toothpick traversing the duodenum and penetrating into the liver. *J Clin Ultrasound* 2006; **34**: 237-240

10 **Horii K**, Yamazaki O, Matsuyama M, Higaki I, Kawai S, Sakaue Y. Successful treatment of a hepatic abscess that formed secondary to fish bone penetration by percutaneous transhepatic removal of the foreign body: report of a case. *Surg Today* 1999; **29**: 922-926

11 **Tsui BC**, Mossey J. Occult liver abscess following clinically unsuspected ingestion of foreign bodies. *Can J Gastroenterol* 1997; **11**: 445-448

12 **Dugger K**, Lebby T, Brus M, Sahgal S, Leikin JB. Hepatic abscess resulting from gastric perforation of a foreign object. *Am J Emerg Med* 1990; **8**: 323-325

13 **de la Vega M**, Rivero JC, Ruiz L, Suarez S. A fish bone in the liver. *Lancet* 2001; **358**: 982

14 **Byard RW**, Gilbert JD. Hepatic abscess formation and unexpected death: a delayed complication of occult intraabdominal foreign body. *Am J Forensic Med Pathol* 2001; **22**: 88-91

15 **Broome CJ**, Peck RJ. Hepatic abscess complicating foreign body perforation of the gastric antrum: an ultrasound diagnosis. *Clin Radiol* 2000; **55**: 242-243

16 **Drnovsek V**, Fontanez-Garcia D, Wakabayashi MN, Plavsic BM. Gastrointestinal case of the day. Pyogenic liver abscess caused by perforation by a swallowed wooden toothpick. *Radiographics* 1999; **19**: 820-822

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CASE REPORT

Carcinosarcoma of the stomach: A case report and review of the literature

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Abstract

Carcinosarcomas are rare, malignant, biphasic tumors. We report the case of a 62-year-old man with gastric carcinosarcoma, along with its clinical, macroscopic and histopathological features. Macroscopically, a specimen of deformed stomach was obtained that measured 200 mm × 150 mm × 100 mm. A 150 mm × 100 mm × 50 mm exophytic tumoral mass (Borrmann type Ⅰ) was found, which involved the posterior wall from the cardia to the antrum. Histopathologically, a mixed type of malignancy was revealed: an adenocarcinoma with intestinal metaplasia, with interposed fascicles of fusiform atypical cells and numerous large, rounded and oval cells. The tumor showed positive histochemistry for cytokeratin 18, epithelial membrane antigen, carcinoembryonic antigen, chromogranin A and vimentin. Liver metastases were diagnosed 8 mo postoperatively, and the patient died 4 mo later. A review of the available literature is also presented.

Key words: Carcinosarcoma; Histochemistry; Pathology; Stomach

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INTRODUCTION

Carcinosarcomas are rare, malignant, biphasic tumors. In the upper gastrointestinal tract, they are most frequently observed in the esophagus, while localization in the stomach has been less frequently reported[1-3]. We present the case of a 62-year-old man with gastric carcinosarcoma, along with its clinical, macroscopic and histopathological features.

CASE REPORT

The patient was a 62-year-old man admitted for surgery with a 2-mo history of blunt epigastric pain, nausea, loss of body weight and intermittent bleeding from the upper gastrointestinal tract. His and his family medical history was unremarkable.

Upon admission, the patient was in a forced position, bent anteriorly with a facial expression of pain. General examination revealed marked pallor, and tenderness in the epigastric region, radiating to the right side of the anterior abdominal wall. In the space of Labbé, an elastic, resistant, fixed mass was palpated. Routine laboratory parameters were found to be normal, except for markers of hypochromic anemia and inflammation: hemoglobin 100 g/L, hematocrit 26%, mean corpuscular volume (MCV) 78 fL, iron blood level 6.8 μmol/L, iron-binding capacity 84 μmol/L, saturation 8%, plasma fibrinogen 6.9 g/L, and erythrocyte sedimentation rate 85 mm at the end of the first hour (Wintrobe). The concentration of CA 72.4 was 110 U/mL.

Endoscopic examination revealed an exophytic, lobulated mass that infiltrated the entire posterior wall of the stomach, from the cardia to the antrum, obturating the gastric lumen throughout. An endoscopically taken biopsy revealed signs of carcinosarcoma, with strongly expressed adenomatous and fibromatous components. Barium-based contrast radiography revealed a satisfactorily passable pyloric canal, despite the initial antral obturation. Computerized abdominal tomography (Figure 1) detected an irregular inhomogeneous, prominent tumorous formation (120 mm × 80 mm × 50 mm) in the stomach, with enlarged solitary lymph nodes of up to 2 cm disseminated along the minor and major gastric curvatures. The patient subsequently underwent total gastrectomy with Roux-en-Y esophagojejunostomy and extirpation of the affected lymph nodes. Macroscopically, a specimen

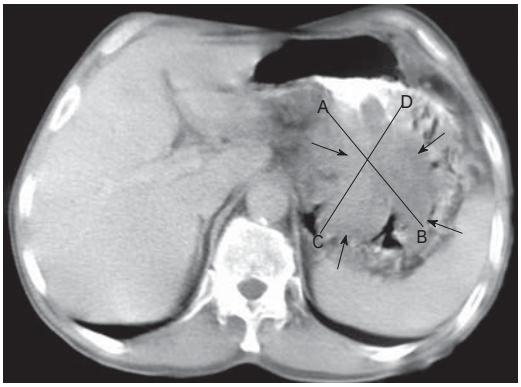

Figure 1 CT scan of the abdomen showing tumor (arrows). AB, CD: tumor diameters.

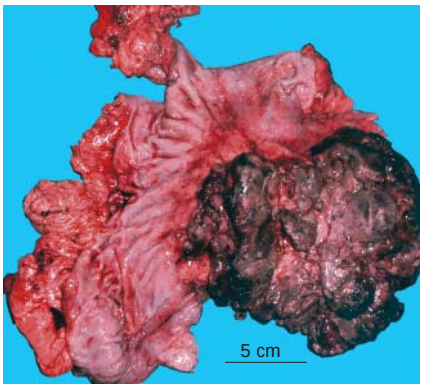

Figure 2 Macroscopic specimen obtained during surgery. Tumor underwent central necrosis, and a hemorrhagic zone is visible on the periphery.

of deformed stomach that measured 200 mm × 150 mm × 100 mm was obtained. A 150 mm × 100 mm × 50 mm exophytic tumoral mass (Borrmann type Ⅰ) was found, which involved the posterior wall from the cardia to the antrum. Areas of necrosis and haemorrhagia were observed in the tumor (Figure 2). The tumor did not invade the esophagus or duodenum, and metastases to other organs were not observed (TNM stage ⅢA).

Histopathologically, the tumor involved all the layers of the gastric wall. The malignancy had two components, epithelial and mesenchymal. The epithelial component consisted of irregular, dilated adenomatous structures, along with a low cylindrical epithelium, with atypical, pleomorphic hyperchromatotic or vesiculous nuclei and detectable nucleoli. Between the glandular formations spread the mesenchymal component, consisting of fascicles of fusiform atypical cells and numerous large, rounded and oval cells, with extremely pleomorphic, hyperchromatic nuclei. Among the aforementioned, multiple atypical, multinuclear giant cells with bizarre hyperchromatotic nuclei and spotted vacuolated cytoplasm were scattered (Figure 3A and B). The epithelial component showed positive histochemistry for cytokeratin 18 (Figure 4), epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA). Chromogranin-A-positive epithelial cells were also observed. The mesenchymal component showed intensive staining for vimentin (Figure 5), although neither muscular nor neural differentiation was found. No *H pylori* was seen.

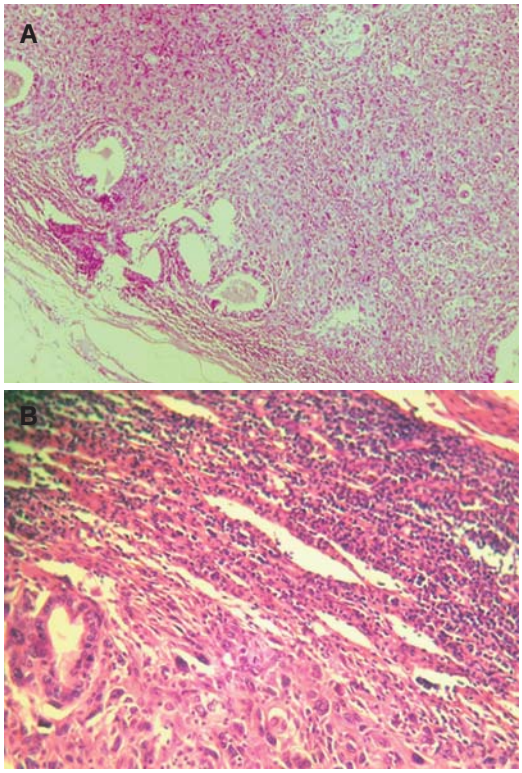

Figure 3 Massive lymph node infiltration with tumor cells. Only a few tubules can be seen on the lymph-node periphery. In between and all around, large polygonal cells are haphazardly arranged (sarcomatous component). This appearance resembles that of the main tumor mass. **A**: hematoxylin-eosin (HE), × 50; **B**: HE, × 100.

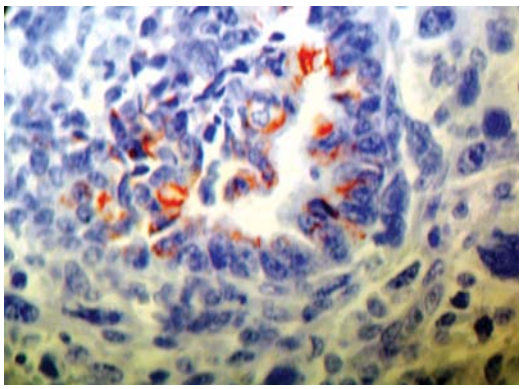

Figure 4 Cytokeratin-18-positive epithelial cells arranged in tubules (× 400).

The patient was discharged on the fifteenth postoperative day in a very well condition. Eight months after operation, liver metastases were observed on CT scanning, but his Karnofsky performance status (50) and Eastern Cooperative Oncology Group performance status (3) did not allow the administration of chemotherapy, and therefore he only received symptomatic medications. He died about 4 mo later.

DISCUSSION

In this paper, we presented the case of a patient with stomach carcinosarcoma, with simultaneous occurrence of moderately to well-differentiated adenocarcinoma

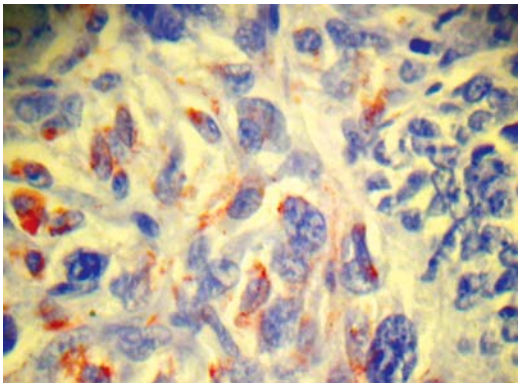

Figure 5 Vimentin-positive polygonal tumor cells (× 400).

and traces of neuroendocrinous, chromogranin-A-positive elements, combined with a vimentin-positive mesenchymal component. To the best of our knowledge, this is the first case of gastric carcinosarcoma seen in this part of the world. According to the available sources, about 50 cases of gastric carcinosarcoma have been reported so far, mostly in Japan and predominantly in the male population, mostly over the age of 60 years[4-6]. An Italian group has experienced five cases of synchronous occurrence of adenocarcinoma and stromal tumor during a 10-year period[7]. Carcinosarcomas in the stomach may be polypoid, exophytic or endophytic, with generally ulcerated surfaces, and they frequently infiltrate the gastric wall in the antral or pyloric region, and form large tumor masses[2,4,7]. Intestinal adenocarcinoma is predominant, but carcinoid and endocrinous or neuroendocrinous elements have been observed during the synchronous appearance of carcinoma and sarcoma in the stomach, while the sarcomatous component can vary between myoblastic, rhabdomyoblastic, chondroblastic and osteoblastic differ entiation[2,5,6,8-11]. Metastasis of carcinosarcoma, however, may be entirely carcinomatous, sarcomatous or biphasic in appearance[4].

Immunocytochemistry seems to be the gold standard for diagnosis of carcinosarcoma, because contrast-based radiography, computerized tomography (CT) and even endoscopy appear to be less efficient, and occasionally, even standard light microscopy is not adequate. Therefore, CEA, EMA, pancreatin, chromogranin A, CD56 and synaptophysin staining are highly specific markers for the carcinomatous components, while desmin, vimentin and α-smooth muscle/sarcomeric actin show affinity for the sarcomatous elements[5,12].

Therapy of carcinosarcoma is always radical and comprises partial or total gastrectomy with Roux-en-Y deviation of one of the jejunal loops, although some complications might appear in the postoperative period[13,14]. Some experimental studies reported possible tumor reduction following treatment with methionine/valine-depleted enteral nutrition, although its efficacy in humans is ambiguous and remains to be established[15].

Prognosis of carcinosarcoma in the stomach is poor[6], and patients with gastric endocrine cell carcinoma have a poorer prognosis than those with other types of gastric carcinoma. The mean survival period is estimated to be 10-15 mo, and overall tumor recurrence in the first postoperative year is greater than 50%[5,9,12].

With respect to the histogenesis of carcinosarcoma, two hypotheses have been proposed. Some authors have suggested that carcinosarcoma is derived from a single totipotential stem cell that has the ability to pursue both epithelial and mesenchymal differentiation[14]. There is no strong evidence that *H pylori* infection influences the appearance of carcinosarcoma[7,14].

In conclusion, carcinosarcoma of the stomach is a rare tumor with high malignant potential, often of unclear etiology. At present, no clinical tests are available for early diagnosis (MRI, barium-based gastrography). The gold standard for definitive diagnosis is immunohistochemical staining of endoscopic biopsy. The possibilities for therapy are confined to radical Roux-en-Y esophagojejunostomy, and recurrence of the tumor can be expected within the first postoperative year.

REFERENCES

1 **Kanamoto A**, Nakanishi Y, Ochiai A, Shimoda T, Yamaguchi H, Tachimori Y, Kato H, Watanabe H. A case of small polypoid esophageal carcinoma with multidirectional differentiation, including neuroendocrine, squamous, ciliated glandular, and sarcomatous components. *Arch Pathol Lab Med* 2000; **124**: 1685-1687

2 **Yamazaki K**. A gastric carcinosarcoma with neuroendocrine cell differentiation and undifferentiated spindle-shaped sarcoma component possibly progressing from the conventional tubular adenocarcinoma; an immunohisto-chemical and ultrastructural study. *Virchows Arch* 2003; 442: 77-81

3 **Insabato L**, Di Vizio D, Ciancia G, Pettinato G, Tornillo L, Terracciano L. Malignant gastrointestinal leiomyosarcoma and gastrointestinal stromal tumor with prominent osteoclast-like giant cells. *Arch Pathol Lab Med* 2004; **128**: 440-443

4 **Kayaselcuk F**, Tuncer I, Toyganozu Y, Bal N, Ozgur G. Carcinosarcoma of the stomach. *Pathol Oncol Res* 2002; **8**: 275-277

5 **Teramachi K**, Kanomata N, Hasebe T, Ishii G, Sugito M, Ochiai A. Carcinosarcoma (pure endocrine cell carcinoma with sarcoma components) of the stomach. *Pathol Int* 2003; **53**: 552-556

6 **Nakayama Y**, Murayama H, Iwasaki H, Iwanaga S, Kikuchi M, Ikeda S, Okada M, Iizuka Y, Iwashita A. Gastric carcinosarcoma (sarcomatoid carcinoma) with rhabdomyoblastic and osteoblastic differentiation. *Pathol Int* 1997; **47**: 557-563

7 **Maiorana A**, Fante R, Maria Cesinaro A, Adriana Fano R. Synchronous occurrence of epithelial and stromal tumors in the stomach: a report of 6 cases. *Arch Pathol Lab Med* 2000; **124**: 682-686

8 **Tsuneyama K**, Sasaki M, Sabit A, Yokoi K, Arano Y, Imai T, Nakanuma Y. A case report of gastric carcinosarcoma with rhabdomyosarcomatous and neuroendocrinal differentiation. *Pathol Res Pract* 1999; **195**: 93-97; discussion 98

9 **Matsui K**, Jin XM, Kitagawa M, Miwa A. Clinicopathologic features of neuroendocrine carcinomas of the stomach: appraisal of small cell and large cell variants. *Arch Pathol Lab Med* 1998; **122**: 1010-1017

10 **Pase F**, Galassi A, Tormen D, Missaglia C, Petrelli G, D'Amore ES. Composite tumour of the stomach: a case report and review of the literature. *Chir Ital* 2005; **57**: 99-102

11 **Kuroda N**, Oonishi K, Iwamura S, Ohara M, Hirouchi T, Mizumo K, Miyazaki E, Enzan H. Gastric carcinosarcoma with neuroendocrine differentiation as the carcinoma component

and leiomyosarcomatous and myofibroblastic differentiation as the sarcomatous component. *APMIS* 2006; **114**: 234-238

12 **Sato Y**, Shimozono T, Kawano S, Toyoda K, Onoe K, Asada Y, Hayashi T. Gastric carcinosarcoma, coexistence of adenosquamous carcinoma and rhabdomyosarcoma: a case report. *Histopathology* 2001; **39**: 543-544

13 **Chen YP**, Yang JS, Liu DT, Chen YQ, Yang WP. Long-term effect on carcinoma of esophagus of distal subtotal gastrectomy. *World J Gastroenterol* 2004; **10**: 626-629

14 **Liu SW**, Chen GH, Hsieh PP. Collision tumor of the stomach: a case report of mixed gastrointestinal stromal tumor and adenocarcinoma. *J Clin Gastroenterol* 2002; **35**: 332-334

15 **He YC**, Cao J, Chen JW, Pan DY, Zhou YK. Influence of methionine/valine-depleted enteral nutrition on nucleic acid and protein metabolism in tumor-bearing rats. *World J Gastroenterol* 2003; **9**: 771-774

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CASE REPORT

Perivascular epithelioid cell tumor of the liver: A report of two cases and review of the literature

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Abstract

Perivascular epithelioid cell tumor (PEComa) is a rare tumor which arises from mesenchymal tissues. It is predominant in the uterus, but very rare in the liver. To the best of our knowledge, less than 5 cases of PEComa of the liver have been reported. Herein we present two pathologically proven cases of PEComa of the liver, retrospectively analyze their clinical and imaging features, and review the literature.

Key words: Liver; Neoplasm; Tomography, X-ray computed; Magnetic resonance imaging

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INTRODUCTION

Perivascular epithelioid cell tumor (PEComa) is a rare tumor which arises from mesenchymal tissues. The uterus is the predominant site, but it is very rare in the liver[1-3]. To the best of our knowledge, less than 5 cases of PEComa of the liver have been reported[2,3]. Herein we present two pathologically proven cases of PEComa of the liver, retrospectively analyze their clinical and imaging features, and review the literature.

CASE REPORT

Case 1

A 56-year-old woman complained of abdominal distention for a week. The laboratory examinations were normal. Ultrasonography found a mass in the superior segment of left lateral lobe of the liver. Non-enhancement CT showed a round isodense mass in the Ⅳ segment of the left liver with ill-demarcated margin. There was no evidence of fatty density, calcification and necrosis in the mass. On contrast enhancement CT, a well demarcated mass, sized 5.1 cm × 4.2 cm, was found with significant and heterogeneous enhancement (Figure 1A). It was more strikingly enhanced on portal venous phase than on arterial phase. Focal nodular hyperplasia (FNH) or adenoma of the left liver was considered before operation. After operation, pathological diagnosis was established as PEComa of the left liver (Figure 1B). Neither primary recurrence nor metastasis was found during the 2-year follow-up.

Case 2

A 63-year-old woman was found to have a mass of liver incidentally in physical examination. Blood, stool and urine routines were normal. Pre-contrast CT scan revealed a lower density mass in the lobus caudatus with a well demarcated margin and homogeneous density. Contrast-enhanced CT showed significantly and heterogeneously patchy enhancement of the lesion on arterial phase (Figure 2A), being slightly hypodense on delayed CT scan. On T1-weighted images, a round homogeneous hypointense mass in the lobus caudatus was found. On T2-weighted images (Figure 2B), the mass had mildly heterogeneous high-signal intensity with a well-demarcated margin. After contrast enhancement, the mass had striking and homogenous enhancement on arterial phase and venous phase. The diagnosis was FNH or HCC of the liver before operation. The gross appearance of the tumor was a smooth, grey and brown lesion with a capsule. The tumor cells were polygonal with eosinophilic cytoplasm. The final diagnosis was PEComa of the liver. Neither primary recurrence nor metastasis could be found during the 1-year follow-up.

DISCUSSION

The term "PEComa" was introduced by Zamboni *et al* in 1996[4]. In 2002 and 2003, two monographs published under the auspices of the World Health Organization (WHO) recognized a family of neoplasm with perivascular epithelioid cell differentiation and accepted the designation "PEComa"[5]. In the WHO soft tissue volume, PEComas are defined as "mesenchymal tumors composed of histologically and immunohistochemically distinctive

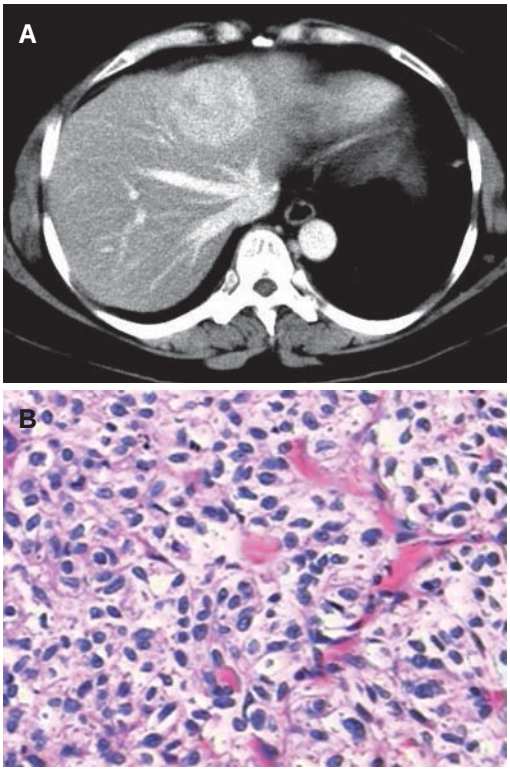

Figure 1 The PEComa of the liver in a 56-year-old woman. **A**: Contrast-enhanced CT scan shows significant and heterogeneous enhancement of the lesion; **B**: Photomicrograph shows polygonal or short spindle cells with oval nuclei and clear abundant cytoplasm (HE, × 200).

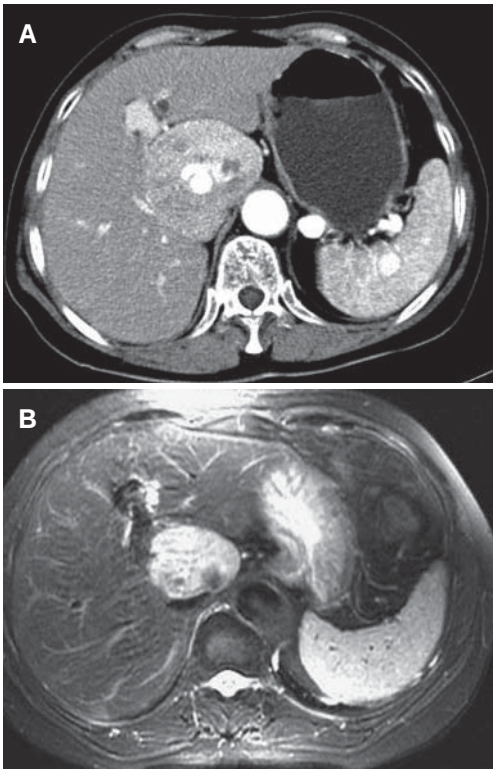

Figure 2 The PEComa in the lobus caudatus of the liver in a 63-year-old woman. **A**: Contrast-enhanced CT scan demonstrates a tumor in the lobus caudatus; **B**: Axial T2-weighted (7058/84) MR image shows a hyperintense mass with well-demarcated tumor margins.

perivascular epithelioid cells (PECs)"[5].

PECs are characterized by perivascular location, often with a radial arrangement of cells around the vascular lumen. Typically, PECs in an immediate perivascular location are most epithelioid and spindle cells resembling smooth muscle are seen away from vessels[5]. The PEC is characterized by positivity with melanocytic markers, such as HMB-45, Melan-A and microphthalmia transcription factor. Desmin is less often positive and cytokeratin and S100 protein are usually absent[5].

PEComas have been reported in the uterus, falciform ligament, gastrointestinal tract, kidney, pancreas, pelvic sidewall, skull, vulva, prostate, thigh, common bile duct and heart. It now appears that PEComas may potentially arise from any anatomic location, but the uterus is the predominant site. Of the 51 cases of PEComa that have been documented in the literature, 46% (21/51) were described in the uterine corpus and 90% developed in females[5]. The PEComas of the liver are extremely rare, and only 5 cases have been reported to date[6].

Clinical presentation of hepatic PEComa has no specificity. It is often found accidentally in physical examinations. But in other organs, the clinical presentation of PEComa might be significant, for example, uterine PEComa may induce uterine bleeding strikingly. Sometimes, a mass can be touched in the PEComa of the lower digestive system and soft tissues.

It is very difficult to make correct diagnosis of PEComa of the liver preoperatively. It is often misdiagnosed as hepatocellular carcinoma, hemangioma, FNH, adenoma and angiomyolipoma. Imaging modalities may be useful because they can help to differentiate PEComa from hepatocellular carcinoma. But the final diagnosis can only be made by pathology.

Clear criteria for malignancy in PEComas have not been elaborated, due to their rarity[5], but there have been a few reports about the tumor metastases[6,7]. On the basis of prior reports, it appears that PEComas displaying any combination of infiltrative growth, marked hypercellularity, nuclear enlargement and hyperchromasia, high mitotic activity, atypical mitotic figures, and coagulative necrosis should be regarded as malignant[5,8]. Malignant PEComas are aggressive sarcomas that frequently result in the death of affected patients, therefore, a close and long-term clinical follow-up is suggested.

REFERENCES

1 **Fang S**, Dong D, Jin M. Perivascular epithelioid cell tumor (PEComa) of the kidney: MR features. *Eur Radiol* 2007; **17**: 1906-1907

2 **Rouquie D**, Eggenspieler P, Algayres JP, Bechade D, Camparo P, Baranger B. Malignant-like angiomyolipoma of the liver: report of one case and review of the literature. *Ann Chir* 2006; **131**: 338-341

3 **Yamamoto H**, Oda Y, Yao T, Oiwa T, Kobayashi C, Tamiya S, Kawaguchi K, Hino O, Tsuneyoshi M. Malignant perivascular epithelioid cell tumor of the colon: report of a case with molecular analysis. *Pathol Int* 2006; **56**: 46-50

4 **Zamboni G**, Pea M, Martignoni G, Zancanaro C, Faccioli G, Gilioli E, Pederzoli P, Bonetti F. Clear cell "sugar" tumor of the pancreas. A novel member of the family of lesions characterized by the presence of perivascular epithelioid cells.

Am J Surg Pathol 1996; **20**: 722-730
5 **Folpe AL**. Neoplasms with perivascular epithelioid differentiation (PEComas). In: Fletcher CDM, Unni KK, Mertens F, editors. Pathology and genetics of tumours of soft tissue and bone. Lyon: IARC Press, 2002: 221-222
6 **Rigby H**, Yu W, Schmidt MH, Fernandez CV. Lack of response of a metastatic renal perivascular epithelial cell tumor (PEComa) to successive courses of DTIC based-therapy and imatinib mesylate. *Pediatr Blood Cancer* 2005; **45**: 202-206
7 **Harris GC**, McCulloch TA, Perks G, Fisher C. Malignant perivascular epithelioid cell tumour ("PEComa") of soft tissue: a unique case. *Am J Surg Pathol* 2004; **28**: 1655-1658
8 **Fadare O**, Parkash V, Yilmaz Y, Mariappan MR, Ma L, Hileeto D, Qumsiyeh MB, Hui P. Perivascular epithelioid cell tumor (PEComa) of the uterine cervix associated with intraabdominal "PEComatosis": A clinicopathological study with comparative genomic hybridization analysis. *World J Surg Oncol* 2004; **2**: 35

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LETTERS TO THE EDITOR

Unusual colonoscopy finding: *Taenia saginata* proglottid

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Abstract

Infection with tapeworms is a major problem in many parts of the world. Patients may be asymptomatic or have a significant morbidity depending on the species. Infection with *Taenia* species is sometimes found by expulsion of eggs or proglottids in stool. Species specific diagnosis of *Taenia* is difficult, but possible. We present a case of *Taenia saginata* incidentally discovered, and risk factors for transmission, diagnosis, symptoms, and treatment.

Key words: *Taenia saginata*; colonoscopy; Tapeworm

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TO THE EDITOR

A 63-year-old Lebanese male presented for routine surveillance colonoscopy of polyps. He denied abdominal pain, hematochezia, weight loss, or change in bowel habits. Physical examination and laboratory studies including a complete blood count were normal. While withdrawing the scope, the following item was seen (Figure 1).

The linear white object represented a parasite. On endoscopic examination, it was found to move within the colon. When retrieved and further analyzed, it was found to be a proglottid of *Taenia saginata*, the beef tapeworm. The scolex and majority of the worm are present in the small bowel, with the head usually residing in the jejunum or ileum. Each segment, known as a proglottid, has a complete set of reproductive organs. The adult worm may have hundreds to thousands of proglottids. The more distal the proglottids are, the more mature they are, containing an increasing number of eggs. *Taenia* species bud off distal segments from the rest of the body that are passed through the feces. The mature *T. saginata* tapeworm can reach 4-6 meters or more in length, and has 1000-2000 proglottids. The scolex has 4 suckers, but no hooks. In contrast, the mature T. solium, or pork tapeworm can reach 2-4 meters or more in length, and has 800-1000 proglottids. The scolex has 4 suckers and a small rostellum with a double crown of 25-30 small hooks[1].

Finding eggs or proglottids in the stool makes the diagnosis of *Taenia* infection. The eggs of *T. saginata* are indistinguishable from *T. solium*, and a species specific diagnosis requires examination of a proglottid segment. The microscopic differentiation of gravid *T. saginata* proglottids (usually more than 15 lateral uterine branches, vaginal sphincter muscle, and two ovarian lobes) and *T. solium* proglottids (usually 5-10 uterine branches on each side, vaginal sphincter muscle absent, one ovarian lobe) is possible. This is the only practical method that can be used in a basic laboratory if only gravid proglottids passed out in stool are present for diagnosis. The presence of a vaginal sphincter muscle in the proglottid can identify the organism as *T. saginata*. The presence of 2 ovarian lobes is also a specific trait of *T. saginata*. Following antihelminth therapy, the scolex is shed and in some cases may be retrieved. The absence of hooks on the scolex is a characteristic of *T. saginata*[2]. If findings are doubtful, the differentiation should be done in a specialized helminthological laboratory by enzyme electrophoresis, polymerase chain reaction (PCR), or various immunological assays[3].

The beef tapeworm is a common infection of both humans and cattle throughout the world, particularly in areas wherever beef is eaten. Areas of high prevalence are sub-Saharan African, southeast Asia, and the Middle East. Infection is associated with eating raw beef, poor sanitation, and allowing cattle on pastures fertilized by sewage sludge contaminated with human feces[4]. Lifecycles for *Taenia* involve two mammalian hosts, a carnivorous or omnivorous host, and a herbivorous intermediate host. In the tapeworm life cycle, humans are the final host and infections are acquired by ingesting raw or undercooked meat containing the cysticercus stage in a host capsule. When the cysticercus stage reaches the stomach, proteolytic enzymes start dissolving the capsule. In the small intestine, the cysticercus is stimulated to evaginate. The scolex attaches to the intestinal mucosa by means of 4 suckers and starts growing into a mature tapeworm.

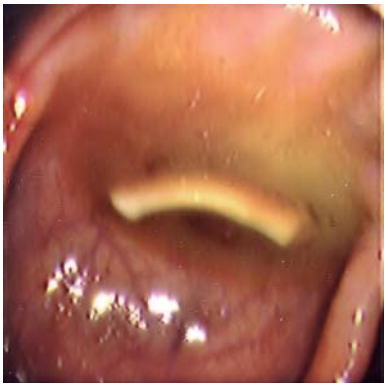

Figure 1 Endoscopic examination showing a parasite (a proglottid of *Taenia saginata*) moving in the colon.

Mature tapeworms have been known to live in the human gastrointestinal tract for up to 25 years[1]. Upon further questioning, our patient noted that he frequently ate raw beef in his native country.

Most patients who carry an adult *T. saginata* tapeworm are asymptomatic. The only symptom found in such a patient may be the spontaneous passage of proglottids. Nonspecific symptoms such as abdominal discomfort, epigastric pain, nausea, vomiting, diarrhea, and weight loss are known to occur[1], but these symptoms are rare. Obstruction of the appendix, pancreatic duct, or common bile duct by proglottid segments has been reported[1]. The occurrence of weight loss and malnutrition is extremely rare. B12 deficiency and megaloblastic anemia are not seen in *Taeniasis*, and associated with *Diphyllobothrium* species found in fish species. B12 deficiency is thought to occur as a result of the ability to compete with the human host for vitamin B12.

While the adult form of *T. saginata* generally does not lead to serious complications, juvenile forms of other tapeworm species may lead to such complications. Ingested ova from *T. solium*, the pork tapeworm, can form cysticercosi in the brain, subcutaneous tissue, skeletal muscle, eye, or other organs causing a significant morbidity. The life cycle of *T. solium* includes pigs that are the intermediate host because they develop the larval stage and transmit the parasite when human beings ingest insufficiently cooked pork[5]. It is endemic in Mexico, Latin America, tropical Africa, southeast Asia, the Philippines, and the Indian subcontinent[2]. Ova from *Echinococcus*, a tapeworm associated with canines, can also cause cysticercosi with a predominance for occurrence in the liver. There is no evidence that cysticerci can develop in humans as a result of T. saginata infection[6].

The treatment of choice in intestinal *Taeniasis* (*T. saginata* and *T. solium*) is praziquantel, a synthetic heterocyclic isoquinolone-pyrazine derivative. A single dose of 5 to 10 mg/kg has an efficacy of greater than 95%. Praziquantel induces ultrastructural changes in the teguments of parasites, resulting in increased permeability to calcium ions[7]. Calcium ions accumulate in parasite cytosol causing muscular contractions and ultimate paralysis of the worm. Additionally, this exposes parasite antigens to the host immune response. The ultimate response is dislodgement of worms from their intestinal sites and subsequent expulsion by peristalsis. For successful treatment, the scolex must be destroyed, and eliminated because residual scolex can result in regrowth. Albendazole or praziquantel can be used in the treatment of cysticercosis.

For people in high risk communities, primary prevention of *Taeniasis* is the removal of intermediate hosts such as cattle and pigs from the parasite's life cycle by eliminating exposure to raw sewage[1] and adequately cooking meats before consumption.

REFERENCES

1 **Collier L**, Cox F, Topley WW. Topley and Wilson's Microbiology and Microbial Infections: Parasitology, Vol 5. London: Edward Arnold Publishers, 1998: 521-537
2 **Murray PR**. Manual of Clinical Microbiology. 7th ed. Washington DC: ASM Press, 1999: 1432-1437
3 **Raether W**, Hanel H. Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections: an update. *Parasitol Res* 2003; **91**: 412-438
4 **Joklik WK**, Willett HP, Amos DB, Wilfert CM. Zinnser Microbiology. 20th ed. Norwalk: Appleton & Lange, 1992: 1208-1210
5 **Flisser A**. Where are the tapeworms? *Parasitol Int* 2006; **55** Suppl: S117-S120
6 **Hoberg EP**. Taenia tapeworms: their biology, evolution and socioeconomic significance. *Microbes Infect* 2002; **4**: 859-866
7 **Hardman JG**, Limbird LE. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw Hill, 2006: 1073-1093

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303]

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7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900]

No volume or issue

9 Outreach: bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

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

Author(s) and editor(s)

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 Tumour 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)

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/eid.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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