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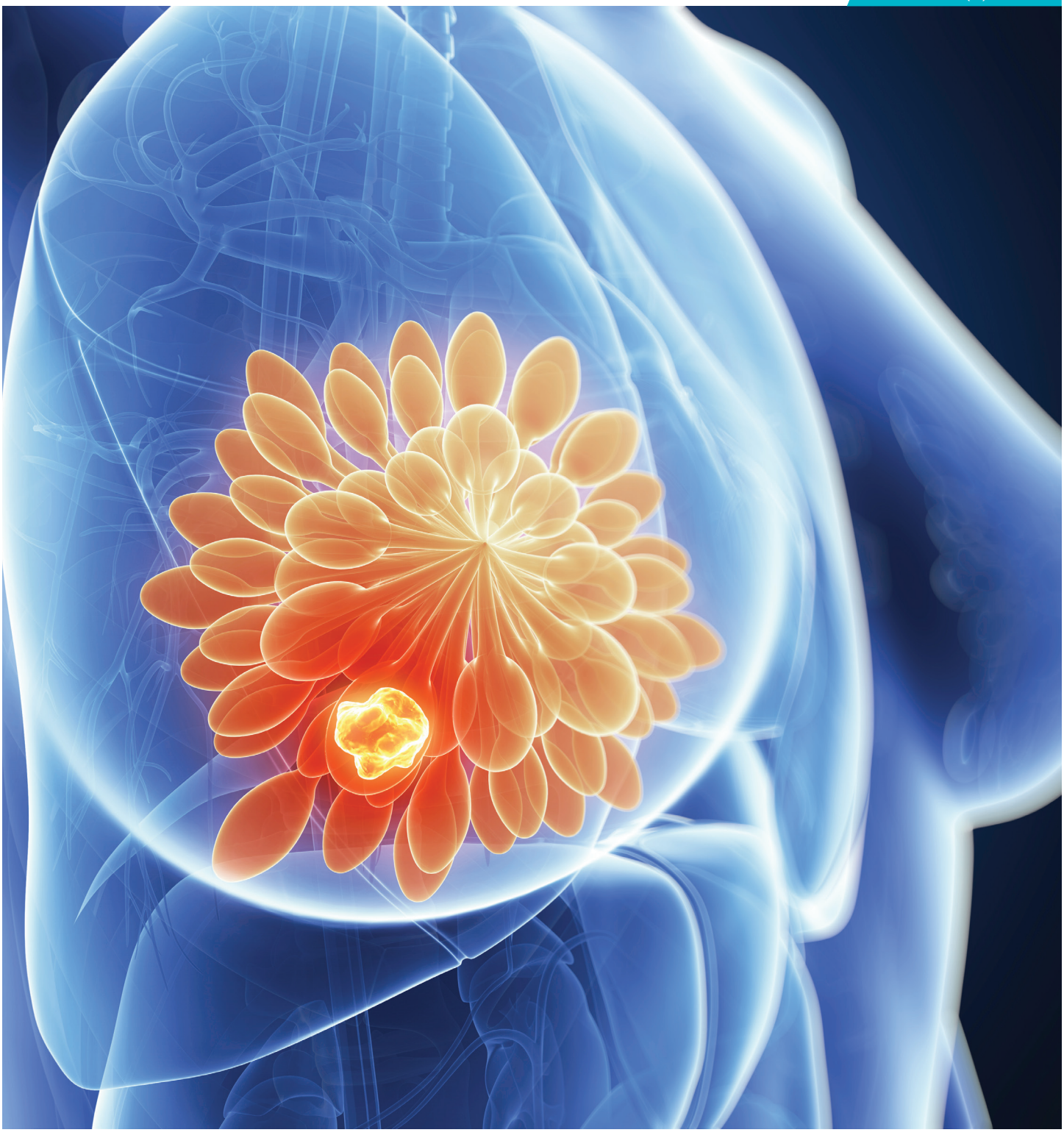

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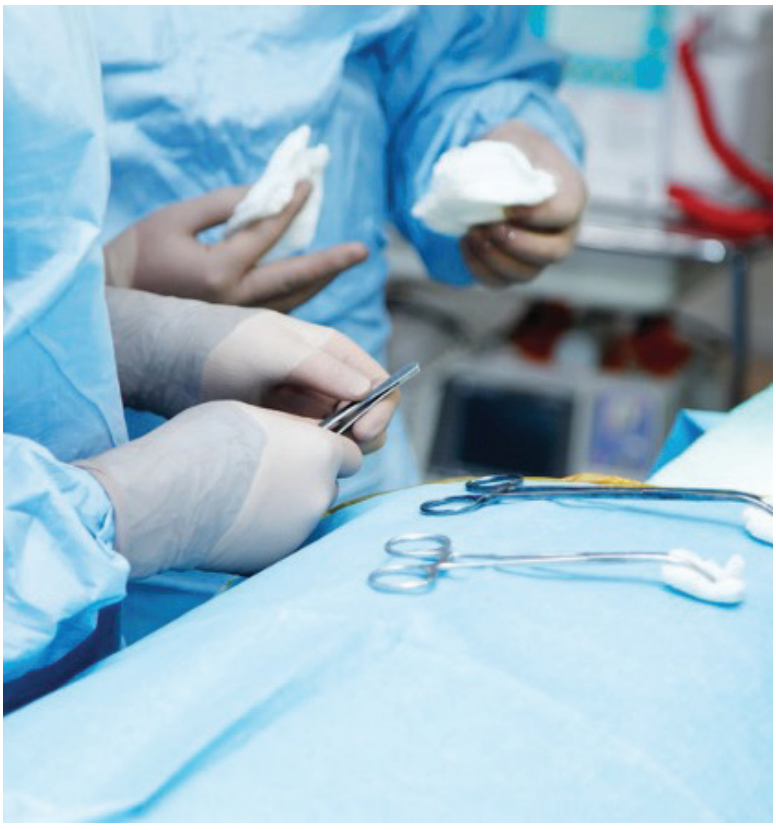

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The key activities of these departments of NSMC are reconstructive surgery, transplantation, innovative minimally invasive approaches, microsurgical correction, cell-based therapy of various surgical diseases.
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JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)
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About the article Morphology of echinococcal liver lesions during treatment with high-intensity focused ultrasound

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Abstract

The authors, after reading the article entitled "Morphology of echinococcal liver lesions during treatment with high-intensity focused ultrasound", published in the Journal of Clinical Medicine of Kazakhstan, were motivated to address the editorial team and the general public to refer their considerations about.

Key words: echinococcosis, hepatic echinococcosis, high-intensity focused ultrasound ablation, environment and public health

Dear Editor

After reading with great interest the article entitled "Morphology of echinococcal liver lesions during treatment with high-intensity focused ultrasound" [1], the motivation arose to refer our considerations in this regard.

We highlight the relevance of the manuscript, since it shows detailed histopathological evidence of the destructive effect of HIFU Ablation (high-intensity focused ultrasound) on mature and germinative forms of echinococcus in the liver. These findings demonstrate a high effectiveness of HIFU ablation and position it as an effective, safe and minimally invasive method in the treatment of hepatic echinococcosis [1].

Hydatid disease (HD) is a zoonosis caused by the larval stages of cestodes belonging to the genus Echinococcus [2], with the liver being the main organ affected (around 75% of cases) [3].

The genus Echinococcus is very broad, however the two main species of medical importance are Echinococcus granulosus which causes cystic echinococcosis (CE) and E. multilocularis responsible for alveolar echinococcosis (AE). Both are serious diseases, especially AE, with a poor prognosis and a high fatality rate if they do not receive adequate treatment [4].

The HD can be asymptomatic for long periods of time, due to slow cyst growth. The usual clinical presentation includes fatigue and abdominal pain. In addition, due to cyst rupture, jaundice, hepatomegaly, or anaphylaxis may occur. Other complications described include rupture of the bile ducts and cholangitis, cystic biliary obstruction, portal hypertension, ascites, intracystic or subphrenic abscesses, and even bronchobiliary fistula [2].

The diagnosis of HD is made with a combination of ultrasound and immunodiagnostic techniques [5].

There are currently pharmacological, conservative and surgical modalities. Medical treatment has low cure rates and high recurrence rates. Conservative procedures with ultrasound controls in which the parasite is sterilized with a scolicidal agent and the cyst is evacuated are associated with high rates of morbidity and recurrence. Radical procedures include liver resections and pericystectomy, with high intraoperative risk and low recurrence rates. The most common postoperative complications are those related to the bile ducts [6].

From the foregoing, it can be deduced that an effective treatment is necessary, one that improves the quality of life of patients with HD with the minimum possible number of complications and unfavorable sequelae.

As in other regions of the planet, EC is a major public health problem in South America, where there is a high underestimation of the disease, which, like other neglected conditions, suffers from serious underreporting (close to 2,000 cases new estimates per year), however some sources estimate that its true incidence could be 2 to 100 times higher than that reported [7]. In Ecuador, cystic echinococcosis is a growing problem related to intensive agriculture and its associated effluents, however it is important to highlight that the data is limited and the prevalence at the national level is unknown [8].

The heterogeneity of the problem, as well as the structural and social determinants of Ecuador and other South American countries, directly influence the treatment of hydatid disease (HD) where traditional treatment modalities are not exempt from complications and high costs [9], as well as control measures have provided modest results [10].

In this sense, we highlight the development and advent of minimally invasive techniques such as HIFU Ablation, which represents a significant advance in the treatment of this disease. We are aware that the extension and replication of techniques such as HIFU Ablation to other regions of the globe, such as the South American continent, would represent a breakthrough with great impact on the quality of life of affected patients and on the understanding of this zoonosis.

On the other hand, this poses certain challenges in terms of public health that should be a call to government action in countries with a prevalence of Echinococcosis. These challenges include the acquisition of the necessary equipment to carry out such procedures, as well as the corresponding training of the professionals in charge, especially in countries with limited access to health resources.

Finally, we highlight the preventive public health actions necessary for the prevention of echinococcosis

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Osteoarthritis: A contemporary view of the problem, the possibilities of therapy and prospects for further research

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Abstract

Osteoarthritis is a chronic degenerative disease characterized by the destructive changes in the articular cartilage, synovitis, subchondral bone sclerosis and osteophyte formation. Today it is the most common joint disease and one of the main causes of disability of elderly people.

This review provides an overview of advances in understanding of osteoarthritis etiology, pathogenesis, histopathology, as well as the results of up-to-date research of the molecular mechanisms underlying this heterogeneous age-related disease at the clinical and fundamental levels.

The article is devoted to a comprehensive review of the osteoarthritis problem, compiled considering the classical understanding of morphological changes, clinical picture, diagnostic methods, and current therapy protocols, supplemented by the modern trends of world research with the prospect of further development and implementation of the latest therapeutic methods, such as nerve growth factor-inhibitors, fibroblast growth factor-18 and stem cells treatments.

Key words: osteoarthritis, pathogenesis, therapy, up-to-date research, biological therapy

Introduction

Osteoarthritis (OA) is the most common chronic disease of the musculoskeletal system, characterized by defective integrity of articular cartilage, subchondral sclerosis, biochemical and biomechanical changes in the extracellular matrix, resulting in the injury of knee, hip, small joints of the hands and spine [1]. According to the generally accepted classification OA may be distinguished between primary (idiopathic) and secondary, which includes post-traumatic OA, metabolic OA (ochronosis, hemochromatosis), endocrine (acromegaly, hyperparathyroidism), neurological and others.

In the global aspect OA represents a significant public health challenge. Being a chronic joint disease OA of the hip and knee is a leading cause of disability in elderly. Moreover, it is the third most rapidly rising disease after diabetes and dementia [2]. With an aging population and an increasing prevalence of obesity worldwide, the burden of OA will continue to rise as the burden on health systems increases [3]. There is evidence of an increased risk of mortality in connection with OA, which is possibly associated with the development of hypodynamia, metabolic and psycho-emotional disorders, with the background of persistent pain syndrome and low-intensity inflammation, that increases the risk of cardiovascular catastrophes [4].

Etiology and pathogenesis

OA is a clinically heterogeneous disease with the multifactorial etiopathogenesis. The most well-known risk factors for OA include overweight, chronic micro-traumatization of the cartilage due to high physical exertion, trauma, metabolic disorders and hereditary predisposition [5]. Older age (above 65 years), woman gender, occupation with overloading of joints, intoxication (smoking, alcohol, uncontrolled medication and heavy metal salts), endocrine and congenital disorders of structure of joints play a role in OA development as the etiological factors.

The pathogenesis of OA is based on degenerative-

dystrophic damage to the articular cartilage, which develops because of imbalance between anabolic and catabolic processes in cartilage and subchondral bone [6]. Articular cartilage consists of a matrix and chondrocytes embedded in it. The extracellular matrix contains proteoglycans and collagen, the content of which decreases in OA, while the structure and biomechanical properties of cartilage are impaired [6]. An impairment of proteoglycans metabolism leads to a violation of the stability of collagen fibers, followed by dehydration and disorganization of the cartilage. The loss of glycosaminoglycans (particularly chondroitin sulphate, as well as hyaluronic acid) leads to a decrease in matrix resistance to the physical stress, and an increase in the sensitivity of the cartilage surface to damage. There is a synthesis and excessive local release of metalloproteinases by chondrocytes, which leads to a progressive slowdown in cartilage repair, an imbalance between the synthesis and degradation of collagen fibers and proteoglycans of cartilage [6]. All together these results in softening, fibrillation, ulceration, and loss of articular cartilage.

The rate of OA cases increases with the metabolic syndrome (MetS) characterised by obesity, elevated level of plasma glucose and triglycerides, reduced the high-density lipoproteins, and hypertension [7]. All these factors have been shown to implicate in the pathogenesis of OA. MetS is a low inflammatory state responsible for glucose and lipid dysregulation leading to increase in the expression of proinflammatory factors and degradative enzymes, lipid deposition in chondrocytes and ectopic inhibition of cartilage matrix synthesis. The increased level of miR-140, miR-27b and hsa-miR-148a caused by pro-inflammatory environment might affect type II collagen and proteoglycan formation [7]. Hyperglycemia contributes to oxidative stress and increase of glycation end-products leading to cartilage damage. Hypertension with the contraction of small vessels could cause insufficient blood supply compromise nutrient exchange in articular cartilage and potential activation of autophagy that leads to cartilage deterioration [7]. Hypertension probably leads to decrease in synthesis of the synovial fluid, which is necessary for cartilage metabolism. Moreover, ischemia can contribute to microstructural changes of subchondral bone leading to initiation of OA [7].

Currently, the concept of the pathogenesis of OA has undergone some changes: it is no longer considered as only a "degenerative disease", but a disease of active biomechanical and cellular processes with chronic low-intensity inflammation that acts as one of the most important factors in the progression. IL-1 and tumor necrosis factor-α (TNFα) contribute to systemic inflammation, which leads to the activation of NF-κB signaling in both synovial cells and chondrocytes [8]. A wide range of pro-inflammatory cytokines IL-1, IL-8, IL-17, IL-6, TNFα, the release of free radicals (NO), and transforming growth factor β (TGF-β) contribute to the progression of OA, synovitis and changes in the viability and function of chondrocytes [9]. Up-to-date studies have shown that systemic inflammation can reprogram chondrocytes via inflammatory mediators towards hypertrophic differentiation and catabolic reactions through the activation of NF-κB, oxidative phosphorylation, and autophagy mechanisms [10]. Inflammation usually begins in the synovial membrane of the joint; the biochemical composition of the synovial fluid is disturbed, the loss of hyaluronic acid and degenerative changes in cartilage increase. Thus, the damage of the main structural components of cartilage such as the connective tissue matrix and chondrocytes results in the initial stage to cartilage degeneration, and subsequently changes in the subchondral bone: sclerosis and eburnation of the subchondral bone, formation of osteophytes and subchondral cysts (Figure 1) [6].

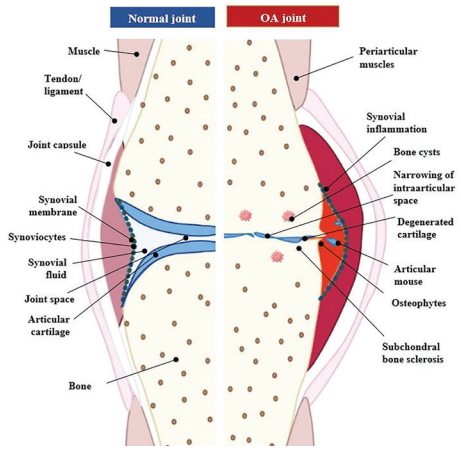

Figure 1 - Illustration of osteoarthritis features with respect to healthy joint. Morphological characteristics of OA joint include reduced joint space due to loss of articular cartilage, low-grade synovitis, hypertrophic reaction (sclerosis) in the subchondral bone following by new bone formation (osteophytes), detached fragment of the cartilage displacement to the articular cavity (articular mouse), inflammation of the synovial membrane and weakness of periarticular muscle and ligaments. Created with BioRender.com.

Morphology

Morphological changes in OA include decrease in the joint space due to loss of articular cartilage, hypertrophic reaction in the subchondral bone (sclerosis), new bone formation (osteophytes) at the joint margins, low-grade synovitis with hyperplasia of the synovial membrane, meniscal degeneration, periarticular muscles and ligaments weakness (Figure 1). In the last stage it may be seen an "articular mouse", the detached fragment of the cartilage displaced to the articular cavity. Additionally, the meniscus in OA becomes thinner and damaged.

Diagnosis

Gradual onset of pain, crepitus during movement due to a violation of the congruence of the articular surfaces, limitation of active and passive movements in the joint, atrophy of the surrounding muscles, as well as deformity of the limbs (varus deformity of the knee joints, Heberden's and Bouchard's nodules) with moderate change in laboratory parameters allow to establish the diagnosis of OA.

X-ray is the routinely used examination, golden standard for OA diagnosis that allows to reveal the narrowing of the joint spaces, osteosclerosis and marginal osteophytes (Figure 2) [11].

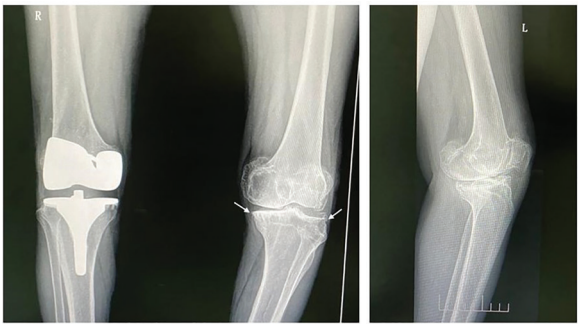

Figure 2 - Typical X-ray changes associated with osteoarthritis. Osteoarthritis of the left knee with narrowing of intraarticular space, hypertrophic reaction of subchondral bone (sclerosis) and new bone formation (osteophytes at the joint margins; labelled with white arrows), accompanied with severe systemic osteoporosis. Patellofemoral arthritis: degenerative changes underneath the kneecap. Total right knee replacement due to decompensation of osteoarthritis with severe pain syndrome and dysfunction of the joint.

Magnetic resonance imaging (MRI) scan permits visualization of a reactive bone oedema, intra-articular structures and inflammation of soft tissue, degenerated cartilage, or articular mouse in the joint. Computed tomography (CT) can be used for the evaluation of menisci and anterior cruciate ligament if needed for clinical decision [11].

There are no obvious laboratory indicators of OA in casual clinical practice, however, studies demonstrated the new molecules that may be used. Maghbooli *et al.* (2019) showed significant 23% decrease in serum levels of complement-C1q TNF-related protein 3 (CTRP3) in postmenopausal women in comparison to age-matched controls and suggested that this could be a clinical marker for osteoarthritis [12]. CTRP3 potentially play a role of anti-inflammatory mediator and may works for prevention of OA and reparation of knee cartilage [12].

The main directions of OA therapy

Classical therapy for OA is aimed at reduction of pain, improving the functional state of the joints, and preventing further destruction of cartilage. Treatment recommendations can be conditionally divided into non-pharmacological, pharmacological and surgical [13]. Among the guidelines available for the treatment of OA the most powerful are the global guidelines developed by the Osteoarthritis Research Society International (OARSI) and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) updated in 2019.

Both OARSI and ESCEO recommend training, structured exercise, and weight loss as the primary treatment option [13]. The data obtained from the first international survey of OA patients the Global OA Patient Perception Survey revealed that more than 80% of OA patients have comorbidities, especially hypertension, and obesity [14].

The association between metabolic syndrome, type-2 diabetes and OA highlighted the need of low cholesterol dietary with increased consumption of long-chain omega-3 fatty-acids [15]. Benefit of patient's ability to self-manage their condition with diet and exercise was proven and included in OA recommendations. A special role is played by non-drug treatment: patient education aimed at ensuring that patients understand the disease, the need for physical exercises that support the function of the joint, and the use of special devices (kneepads, orthopedic insoles, orthoses and canes) for unloading joint [16]. It is necessary to achieve compliance between a patient and a doctor, the patient understanding that moderate physical exercises (such as swimming, cycling, walking) contribute to reducing of pain and improving the functional activity of the joints [16].

Pharmacological therapy includes the use of paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs), the choice of which should be made individually and dictated primarily by a safety assessment. The most serious complications of this therapy are expected from the gastrointestinal tract, and COX-2 inhibitors have the lowest risk; their appointment is justified in a group of patients over 60 years of age, with concomitant diseases and a history of gastrointestinal tract pathology. According to the latest OARSI recommendations, topical NSAIDs are recommended for use in the first line of OA treatment, if ineffective - paracetamol. According to the recommendations of OARSI and ESCEO, paracetamol and NSAIDs should be used only during the period of increased pain syndrome [17]. For persons with concomitant cardiovascular diseases, the use of any oral NSAID is not recommended [13].

The OARSI guidelines for the non-surgical management of knee, hip and polyarticular osteoarthritis emphasize the appropriate use of intra-articular injections of corticosteroids and hyaluronic acid, as well as the need for aquatic exercise for the treatment of OA [17]. The OARSI and ESCEO guidelines support the use of intra-articular corticosteroid injections in patients with persistent pain unresponsive to topical and oral NSAIDs [13].

Hyaluronic acid drugs for intra-articular administration are indicated in both guidelines [13]. The use of hyaluronic acid is recommended for patients with contraindications to NSAIDs or persisting pain syndrome despite taking NSAIDs. These drugs are well tolerated and have small analgesic effect. Recent studies of the effectiveness of hyaluronic acid injections in patients with osteoarthritis of the knee joint showed a decrease in the severity of pain syndrome according to the visual analogue scale (VAS) and an improvement in joint function according to the Leken index [18].

Symptomatic slow-acting drugs for osteoarthritis (SYSADOA), including chondroitin and glucosamine sulphate, as well as their combination, avocado soybean unsaponifiables and diacerein, are well tolerated by patients and can help improve the effectiveness of treatment and reduce possible functional impairment [19]. Meta-analyses of placebo-controlled trials of SYSADOAs treatment demonstrated evidence of safety and small beneficial effects in patients with OA [19, 20]. OARSI and ESCEO recommendations are divided regarding glucosamine and chondroitin sulphate: they are recommended by ESCEO, while OARSI does not recommend their use, considering them ineffective [13].

Surgical treatment includes arthroplasty, which is prescribed for patients with severe joint dysfunction accompanied by intractable pain syndrome [21]. Lavage of the knee joints with removal of detritus and arthroscopic removal of the "articular mouse" can also reduce pain but does not stop the disease progression [21].

COVID-19 and OA

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), became an important issue for healthcare in the worldwide for a few years. It affected different organs and systems and may potentiate development of a range of autoimmune disease, including arthritis, however, there is a lack of data about OA cartilage degeneration due to COVID-19 [22]. Endothelial and adipose tissue dysfunction induced by SARS-CoV-2 through its influence on angiotensin-converting enzyme 2 (ACE2), adiponectin concentration, and apoptosis lead to a meta-inflammation [22]. Acute pain is associated with the production of pro-inflammatory cytokines, including CCL2/3/4, CXCL2, IL-1, IL-6 and TNF [22]. Hypovitaminosis D, hypocalcaemia followed by demineralization and bone fragility in patients with COVID-19 point towards bone aging, while arthralgia and myalgia resemble OA aging characteristics and may be classified as early OA-like phenotype of COVID-19 [22].

COVID-19 negatively influences on patients with chronic diseases including OA. Delay of nonemergency procedures due to COVID restrictions lead to a significant number of postponed joint replacement operations or cancellations of surgical or therapeutical procedures [23]. COVID control have reduced access to healthcare services most of them became through remote working (telemedicine, telehealth, virtual consultations) [24].

OA usually going with comorbidities such as cardiovascular disease, diabetes, and obesity, that increase the risk of COVID-19 infection and its severity. Restrictions of access to exercise facilities may lead to limitations in range of motion,

hypodynamia and subsequent muscle atrophy of individuals with OA. So even in COVID quarantine patients are strongly advised to continue to exercise near or at home (walking, yoga, tai chi) and reduce weight with a healthy diet [23].

The question about routinely prescription of NSAIDs in OA was highly debatable. Research revealed no negative effect of prescribed NSAIDs on COVID-19 related deaths [24].

New therapeutic opportunities and prospects for further research

Throughout the history of OA research several therapeutic approaches have been proposed (pharmacological treatment, physiotherapy, acupuncture), but none of them leads to a complete cure or even to reliable pain relief and improvement joint function. The absence of effective disease-modifying therapy induces a vast of OA research worldwide.

Intra-articular platelet rich plasma

Intra-articular platelet rich plasma (PRP) appears to be a promising therapy in the last few decades. PRP preparations are prepared by separating autologous blood (about 100 ml) by a two-stage centrifugation method, which separates plasma and cellular elements. The concentration of platelets in this preparation is about 1 million/μl, which is about 5 times higher than the content in native blood. Platelets are activated by adding thrombin or fibrin "matrix", and then the resulting clot ("gel") is injected with a syringe into the affected area. Platelets secrete platelet growth factor, TGF-β, fibroblast growth factor, insulin-like growth factor 1 and 2, which act as tissue hormones and factors of migration and differentiation of stem cells [25]. Some authors have noted a significant reduction in pain after PRP compared with baseline in patients with knee OA, regardless of age, sex, severity and body mass index [26]. The combination of platelet-rich plasma with hyaluronic acid administered intra-articularly to patients with OA showed the same efficacy as PRP alone [27]. However, intra-articular PRP did not show a significant advantage over placebo to add this method to the recommendations for the treatment of knee OA [28, 29].

Cellular therapy

Cell therapy methods in the treatment of OA are being widely researched. Mesenchymal stem / stromal cells (MSCs) have gained significant popularity due to the enormous opportunities, lack of ethical limitations and risks usually associated with other stem cells such as embryonic stem cells. MSCs have been identified in the synovial fluid of healthy people; however, with arthritic changes, the number of MSCs increases significantly [30, 31]. Jones et al (2008) found that the level of MSCs obtained from synovial fluid is seven times higher in OA compared with the control group [32]. It was known that MSCs can play a positive role in the restoration of cartilage tissue in the pathogenesis of arthritis. De Sousa et al (2014) emphasize that these cells maintain homeostasis, involved in the restoration of joint tissue and restore the balance between catabolism and anabolism of cartilage tissue [33]. MSC studies have demonstrated cartilage remodeling in a mouse model of OA, as well as a decrease in pain due to damage to the subchondral bone when using the MSC secretome. After preclinical evaluation in experimental animal models, MSCs began to be used in single studies in patients with OA. However, according to the Canadian recommendations for intra-articular injections for osteoarthritis of the knee, there is currently insufficient evidence to recommend MSCs for the treatment of OA [29]. Thorough, well-planned clinical trials are required to establish the safety, efficacy, and cost-effectiveness of MSCs before including in the treatment protocols.

A promising method for the treatment of OA may be induced pluripotent stem cells (iPSCs). Patient-specific stem cells can be created by reprogramming a somatic cell to a pluripotent state, for example, by transferring its nucleus to an oocyte. SOX2 and OCT4 in combination with KLF4 and cMYC also promote the reprogramming of human fibroblasts into iPSCs. These studies demonstrated that pluripotency can be restored in a terminally differentiated cell, and suggest that these cells will be able to support the infinite production of functional chondrocytes [34].

Autologous chondrocyte transplantation was proposed as a variant of cell therapy for OA in the recent years and shown to be effective in restoring hyaline cartilage [35].

Monoclonal antibodies

One of the main therapeutical directions recently explored is monoclonal antibodies, such as anti-TNFα agents (adalimumab, infliximab and etanercept) and IL-1 inhibitors (anakinra, canakinumab), which are successfully used for rheumatoid arthritis, psoriatic, enthesitis-related and juvenile idiopathic arthritis treatment [36, 37]. However, they did not demonstrate statistically considerable efficacy in OA: adalimumab did not significantly decrease a pain, synovitis or bone marrow lesions in patients with erosive hand OA [38]. IL-1 targeting therapies also failed to bring remission in OA: IL-1α/β immunoglobulin had no effect on pain or imaging outcomes in patients with erosive hand osteoarthritis [39] and minimal decrease in pain score with no improvement in synovitis of knee compared to the placebo group [40]. Data about IL-6 involvement in OA pathogenesis promoted OA research with tocilizumab, an antibody against IL-6 receptor, though no efficacy was found in 83 patients with hand OA [41].

Biological therapy also includes the antibodies against nerve growth factor (NGF) [42-44]. Nerve growth factor (NGF) is the major mediator of pain, binds to tropomyosin receptor kinase A and p75 on nociceptive neurons [45]. Taking into account the key role of pain in OA and the side effects associated with long-term use of NSAIDs and opioid analgesics, anti-NGF antibodies became a promising therapeutical tool for OA treatment. Anti-NGF monoclonal antibodies currently include 3 medications (tanezumab, fasinumab and fulranumab). The drugs are currently under III phase clinical trials, but promising clinical results such as pain relief and physical function improvement have already been obtained [42, 43]. However, despite the high expectation the side effects of these drugs have to be carefully analysed before anti-NGF can enter the therapeutic arsenal for OA.

The search for the disease-modifying drugs for OA continues, and fibroblast growth factor (FGF)-18 may take this position. FGF-18 is known as a molecule that protects articular cartilage due to anti-catabolic effects mediated by tissue inhibitor of metalloproteases (TIMP)-1, stimulation of chondrocytes and maintenance of cartilage homeostasis [46]. Recombinant human FGF-18 (sprifermin) is considered a disease-modifying drug used as an intra-articular injection, and it has been shown to reduce the injury of cartilage [46]. Moreover, it was demonstrated that the intra-articular administration of 100 μg of sprifermin every 6 or 12 months resulted in significant improvement in total femorotibial joint cartilage thickness after 2 years [47].

Others: metformin

Nowadays there are conducted the range of studies dedicated to the effect of various substances that may affect the processes of destruction and formation of cartilage. For example, Li *et al.* (2020) conducted an experimental study of the effects of metformin on cartilage in a mouse model of

osteoarthritis. It is known that metformin can induce adenosine monophosphate-activated protein kinase, which is postulated as a potential therapeutic target for the treatment of OA. In vitro experiments have shown that metformin not only lowers the level of matrix metalloproteinase 13, but also increases the production of collagen type II, thereby reducing the severity of structural damage in OA and reducing pain [48].

Further directions

A number of studies have been carried out using large-scale genome-wide screening of miRNAs expressed in osteoarthritic cartilage or subchondral bone, microRNAs (miRNAs) have been identified, which play an important role in cartilage homeostasis and the OA process and can potentially be used to modify the disease [49]. miRNAs are short non-coding RNAs (18-14 nucleotides) that bind to one or more mRNAs to regulate their expression by inhibiting the translation or increasing mRNA degradation. Most of the published studies focused on one or two miRNAs and are based on the hypothesis that they target the gene that plays an important role in the pathogenesis of OA. For example, miR33a regulates cholesterol metabolism in chondrocytes via the TGF-β1/Akt/SREBP-2 pathway, while ABCA1 and ApoA1 genes associated with cholesterol efflux [50]. It was found that MiR-370 and miRNA-373 regulate the expression of SHMT-2 and MECP-2 in chondrocytes [51]. MiR-16-5p has been shown to regulate the expression SMAD5 in cartilage [52], while miRNA-26a-5p regulates the expression of inducible nitric oxide synthase (iNOS) by activating the NF-κB pathway in chondrocytes in OA [53]. The expression of osteopontin in cartilage is regulated by miR-127-5p [54], while miR-139 inhibits the proliferation and migration of chondrocytes [55]. The results of these studies may contribute to the development of radically new methods of therapy for OA in the nearest future.

Conclusion

Despite the huge amount of data of the OA the current treatment options are very limited with no effective cure that might stop or slow down the progression of this disease. The therapeutical options include NSAIDs, paracetamol, non-opioid analgesics, steroids, SYSADOAs, non-pharmacological and surgical methods. In recent years, significant progress has been achieved in understanding of the pathogenetic mechanisms of OA and promising options for biological and cell therapy have been proposed. However, further clinical studies are needed to examine their safety and efficacy.

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Assessment of quality of life one year after in COVID-19 cases using the SF-36

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Abstract

One of the features of COVID-19 infection is a long recovery process and development of the long-term health effects of COVID-19. Therefore, the interest of scholars in ensuring patients' quality of life after treatment of COVID-19 is increasing and puts a long-term health assessment on the agenda. However, there have been limited studies examining subjective evaluation of physical and mental health of patients who have undergone COVID-19 in Kazakhstan.

The study aims to examine the subjective health assessment of patients who suffered from COVID-19 in 2020 and 2021 in Nur-Sultan city using the SF-36 tool. These patients were included and observed in the research with confirmed and probable COVID-19 cases as well as their close contacts.

Material and methods: The study employed questionnaires of respondents through direct interviews, including common questions SF-36. The scoring was done in Microsoft Excel. Statistical analysis of data was performed using the SPSS program, version 23.

Results: Questionnaires were administered among 64 out of 172 patients, 52 (81%) were women and 12 (19%) were men. The majority of respondents were over 40 (41%) and 31 (31%) years old. Nearly half of participants (46%) responded that their health condition was about the same as a year ago, 27% rated their health somewhat worse than a year ago, and 2%, that is, 1 participant, rated their condition as much worse than before COVID-19. Men considered themselves significantly healthier than women ($p>0.05$).

Conclusion: There is a need for additional research on "Long COVID-19" using more specific HRQoL instruments.

Key words: COVID-19, quality of life, outcomes, SF36, health **evaluation**

Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by the rapid spread of coronavirus (SARS-CoV-2) among people via droplets that contain the virus [1]. In the early stages of the disease, patients often develop severe pulmonary pneumonia, and acute respiratory distress syndrome that can lead to severe consequences including multiple organ failures [2]. In addition to the pulmonary system, COVID-19 can affect many other organ systems, including the neurological (anosmia, consequences of thromboembolic events such as stroke, cognitive impairment), cardiovascular (damage to the heart muscle, heart failure), also cause anxiety, depression, sleep disorders, musculoskeletal problems and fatigue [3]. One of the features of COVID-19 coronavirus infection is the long recovery from the disease so called "Long/post COVID-19" that characterizes with high fatigue, shortness of breath, cough, sleep disturbance, muscle pain, depression, etc. and may last from few weeks to years. According to the World Health Organization, 10-20% of the population develops medium- or long-term effects after suffering COVID-19 [4]. Furthermore, a systematic review of articles shows that 59% of patients with "Long COVID-19" rate their health status as poor, with predominant symptoms of high fatigue, shortness of breath, sleep disturbances, and psychological disturbances [5], which directly affect their physical activity and performance capacity. Therefore, the interest in ensuring the quality of life after undergoing COVID-19 is increasing in the academia that makes the continuous health assessment of COVID-19 patients essential in subsequent years.

To evaluate health of patients, the health-related quality of life (HRQoL) is commonly used in clinical

practice and public health field that allows to assess subjective feeling of patients concerning the impact of the disease through 36-item questionnaire (SF-36) [6,7]. Most of studies shows that the Long COVID-19 negatively affects both physical and mental HRQoL, however the symptoms and characteristics of "Long COVID-19" vary by country. For example, in Wuhan Province, China, among 2469 patients who had coronavirus infection, 55% had low HRQoL for the next two years, namely patients experienced fatigue, muscle weakness, low physical activity tolerance and psychological health problems. At the same time, 89% of patients who underwent COVID-19 returned to their original job only after two years. Nevertheless, overall health at two years was significantly worse in those with severe COVID-19 compared with the general population [8]. A similar pattern was described in Bangladesh, where more than 80% of patients had symptoms of "Long COVID-19" such as fatigue and pain for the next three months after recovery, which limited patients' functional activity [9]. In Turkey, 40% of patients had pain in their joints and lower back for the next three months after recovery [10].

The leading causes of "post-COVID-19" symptoms in the American study were identified as physical exertion, stress, and dehydration. With the appearance of cognitive dysfunction, the patients' level of social activity decreased, which affected their physical activity and performance [11]. Moreover, after entering the workplace, many patients report high fatigue (60-70%) [12]. Only 10.8% of patients in Egypt noted that they had no symptoms after suffering COVID-19, whereas the majority responded that there was prolonged fatigue. In rare cases, patients after recovery from COVID-19 had stroke, renal failure, myocarditis, and pulmonary fibrosis [13]. Thus, most patients develop post-COVID-19 syndrome or "Long COVID-19", which manifests in mild symptoms such as fatigue, muscle pain, and cough, and in a severe form with exacerbation of chronic diseases or complications such as stroke, myocarditis and others.

There is limited literature on examining the effects of coronavirus infection on human health, specifically the subjective assessment of health, both physical and mental, on patients who have undergone COVID-19 in Kazakhstan. The study in Shymkent showed that more than 85% of elderly respondents had post-convulsive complications in the form of increased fatigue, hair loss, myalgia, shortness of breath, fever and headache. Nevertheless, the authors emphasize that a differential diagnosis is necessary since these symptoms cannot always be justified only by the post-covid condition of patients and can manifest during the ageing process and chronic diseases. Notwithstanding, the prolonged character of these symptoms appears more in the category of older adults [14]. Moreover, there is no analysis of patients' subjective self-assessment of their health one year after COVID-19 in Nur-Sultan city. This study aimed to examine the subjective health assessment of COVID-19 patients who were included and observed in a study of confirmed and probable COVID-19 cases and their close contacts in 2020 and 2021 in Nur-Sultan, using the SF-36 questionnaire. Based on the literature review, the study hypothesizes that the health of Kazakhstani population has worsened after recovery from COVID-19. The study limited assess the general state of patients who had COVID-19 infection without specifying the symptoms that persisted or reappeared.

Material and methods:

The study conducted within the international project of the WHO, National Center of Public Health and Astana Medical University "Investigation of confirmed and probable cases of COVID-19 and their close contacts" according to a specially developed protocol approved by the Ethics Committee.

A prospective study included 122 patients with varying degrees of severity and with a confirmed diagnosis of COVID-19 by PCR who were on outpatient treatment in Nur-Sultan, as well as 50 patients who were hospitalized with a clinical picture of COVID-19 but with a negative PCR result and with signs of COVID-19 pneumonia, aged 0 to 90 years. Further, close contacts of confirmed and probable cases were also included in the study. A follow-up of cases and their close contacts was conducted from November 26, 2020 to February 15, 2021.

Sixty-four out of 172 patients participated in the survey. The survey based on subjective assessment of their health conducted one year after the coronavirus infection, in April 2022, according to the study design. Patients were included in the study only after signing the consent to participate in the study.

The exclusion criteria of participants recruitment were patients who died before the start of follow-up, patients whose observation was difficult due to repeated hospitalization or immobilization before or after discharge due to diseases such as stroke or pulmonary embolism and other complications, patients who refused to participate in the study.

The SF-36 questionnaire is commonly used in the medical field to assess the health of individuals or populations by grading eight health dimensions. Due to restriction policy during the conducting data collection, the survey distributed online to collect the data in a safe and effective manner. Respondents filled out questionnaires independently after receiving information about purpose of the research, how the results will be used and the main rules of filling out the SF-36 questionnaire. Standard questionnaire SF-36 has three levels: - 36 questions; - 8 scales. The following scales were analyzed: Physical function (PF), Role Physical (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), Social Functioning (SF), Role Emotional (RE), Mental Health (MN). Thus, the SF-36 questionnaire summarizes two components: physical and mental. The scoring of the scale was from 0 to 100, where 100 represents total health, and the calculation was made using the SF-36 questionnaire scoring methodology in Microsoft Excel.

Before calculating the scores of the 8 scales, the responses were recorded in MS Excel, then the scores were summed to obtain the values of each scale according to the methodology presented in the SF-36 application manual [15]. Equivalent scores are assigned to responses, where 100 is the highest and 0 is the lowest.

Since the majority of the patients from the original study declined or could not be interviewed one year later, the sturdy found it unrepresentative to classify them by disease severity, level of medical assistance or socio-economic status due to the small sample.

Ethical aspects

Before starting the study, all documents, including the research protocol and data collection questionnaires, underwent expert review and received positive approval from the Local Ethics Commission of the NJSC Astana Medical University, Minutes of Meeting No. 9 of 09.09.2020. Patients were included in the study only after they received full information about it and gave written voluntary consent to participate. All information collected concerning the health status of patients is provided with confidentiality in accordance with the Law of the Republic

of Kazakhstan dated May 21, 2013, N 94-V "On Personal Data and Their Protection" and Article 28 of the Code of the Republic of Kazakhstan "On Public Health and Health Care System". Before the study began, the entire research team signed a non-disclosure agreement.

Statistical analysis

Statistical analysis was performed using SPSS software, version 23. The validity and reliability of the SF-36 questionnaire were tested, and statistical parameters such as the mean, standard deviation, and percentage calculation were described.

Results

The survey was obtained by 64 out of selected 172 patients, 108 individuals were unreachable or denied participation, and therefore, there survey response rate was 37%. Since there was limited data on the SF-36 measurements and the study sample was small, this study did not perform an in-depth statistical data analysis. At the time of this survey in average 410 days have passed since the last patient included in the study received a positive PCR result. According the study protocol it was planned to conduct a survey one or a year and a half later to examine long-term health effects after COVID-19.

Fifty-two (81%) women and 12 (19%) men participated in the study. Most respondents were over 40 years old (41%) and 31 to 35 years old (31%). A large proportion of study participants responded that their health conditions about the same as it was a year ago (46%), 27% of respondents assessed their condition as somewhat worse than a year ago, and 2%, that is, 1 participant assessed his condition as much worse than before COVID-19 (Table 1). The highest possible score for a health dimension would be 100, which reflects a very high quality of life in that area. The lowest possible score is 0, which reflects a very low quality of life in that area.

Table 1 Distribution of respondents by gender and age.

Age	Total (N)	Gender composition (% of sample size)	
	Number of respondents (% of the sample size)	Male	Female
Under 25 years old	4 (6,25)	1 (1,56)	3 (4,69)
26-30 years old	2 (3,13)	0 (0)	2 (3,13)
31-35 years old	20 (31,25)	4 (6,25)	16 (25)
36-40 years old	12 (18,75)	2 (3,13)	10 (1,56)
40 and above years old	26 (40,63)	5 (7,81)	21 (32,81)

Table 2 Averages of 8 SF-36 scales (N=64).

SF-36 scales	M	∂
PF	70,78	28,78
RP	69,53	34,64
BP	74,59	21,59
GH	64,95	18,19
VT	56,79	17,71
SF	74,61	17,67
RE	63,54	43,53
MH	58,63	14,77

Bodily Pain (BP), General Health (GH), Mental Health (MN), Physical function (PF), Role Physical (RP), Role Emotional (RE), Social Functioning (SF), Vitality (VT).

The mean values of the eight transformed SF-36 respondents' scales are in Table 2. The mean physical functioning (PF) was at 70.78%, which reflects high physical functioning. The mean role limitations due to physical health (RP) were at 69.53%, where respondents were not enough limited due to physical health. The mean role limitations due to emotional problems (RE) were at 63.5%, where respondents were moderately limited due to emotional problems. Vitality (VT) was at 56.9%, where respondents had moderate vitality levels. Social functioning (SF) was at 74.6%, where respondents were fairly high socially functioning. Body Pain (BP) was at 74.6%, where respondents were in enough pain for a time. General health was at 64% and Mental health was at 58,6% which is mildly-to-moderate.

Validity and reliability analysis

Questionnaire responses were tested with the recommended reliability and validity tests. Internal consistency is the degree to which items within one dimension correlate [16]. We used nonparametric versions of these tests to avoid assumptions about the distribution. The internal consistency was acceptable. Correlations between items and correlations of own measurements, after correction for overlap, did not exceed 0.6 for all items. Cronbach's alpha coefficient did not exceed the recommended minimum of 0.764, and reliability coefficients were above 0.731 for all dimensions except role activity (a=0.725), and vitality (a=0.727) (Table 3).

Table 3 Reliability of the SF-36 questionnaire.

SF-36 scales	Correlation between item and total	Cronbach's Alpha
PF	,468	,738
RP	,543	,725
BP	,379	,752
GH	,547	,733
VT	,603	,727
SF	,572	,731
RE	,436	,774
MH	,592	,735

Bodily Pain (BP), General Health (GH), Mental Health (MN), Physical function (PF), Role Physical (RP), Role Emotional (RE), Social Functioning (SF), Vitality (VT).

Table 4 shows the distribution of SF-36 scale scores by gender and age. The distribution of scores was as expected, indicating the validity of the survey tool. Men considered themselves significantly healthier and scored higher than women ($p>0.05$) on all scales except for the role-activity dimension. Significant results were found for physical functioning and bodily pain ($p>0.05$), but a slight gradient was found for mental health ($p=0.75$). Younger respondents, those under 25 years of age, in contrast to other age groups, scored better on social functioning, pain, and mental health and also presented an excellent gradient on general health ($p>0.05$). Respondents in the 26-30 age group scored highest on emotional state, role functioning, and general health ($p>0.05$) but were vulnerable to bodily pain. Individuals in the 36-40 age group performed well on average on all dimensions except mental health ($p=0.23$) and vitality ($p=0.75$).

The physical and mental health

The physical component was higher among males and was 53.3 (CI 95% 48.4; 54.2). Among females this component was assessed at 47.5 (CI 95% 46.1; 48.9), ($p=0.2$) which is moderate-

Table 4 Average scores on the SF-36 questionnaire dimensions concerning respondent gender and age.

Variables	Number (n)	Physical functioning (PF)	Social functioning (SF)	Role Physical (RP)	Role emotional (RE)	Body Pain (BP)	Mental Health (MH)	Vitality (VT)	General Health (GH)
Age (years)									
< 25	4	80,0	81,9	75,0	41,7	81,9	63,0	61,3	68,5
26-30	2	52,5	75,0	100,0	100,0	69,5	48,0	65,0	69,5
31-35	20	76,5	72,5	67,5	70,0	74,3	58,4	56,3	62,1
36-40	12	85,0	79,2	79,1	63,9	77,0	56,3	54,2	64,8
> 40	26	59,8	73,1	63,5	60,0	73,0	60,0	57,1	66,3
Sex:									
Male	12	79,6	78,1	66,7	69,4	85,7	58,6	60,4	68,4
Female	52	68,8	73,8	70,2	62,2	72,0	58,7	60,0	64,1

to-severe. 11 (55%) individuals assessed the physical component of Physical Health>=50 in the 31-35 age group, one individual (50%) among the 26-30 age group, three individuals (75%) from the under-25 age group, 36-40 age group, six individuals (50%), and nine individuals (35%) among the over-40 age group, (p=0.05).

The mental component was rated almost equally among both sexes at 43.5 (CI 95% 42; 45) among women and 43.7 (CI 95% 40.8; 46.6) among men (p=>0.05). Among age groups, this component was the least pronounced, i.e. Respondents considered themselves vulnerable to the mental stateside. The psychological component was assessed moderate-to-high (PsH>=50) by 8 (40%) persons in the age group 31-35 years, one person (25%) among the age group under 25 years, two persons (17%) among the age group 36-40 years, and six persons (23%) among the age group over 40 years (p=>0.05).

Discussion

The majority of respondents from this study were more or less satisfied with their health, which is indicated in the evaluation scales used in the research that are all relatively good - above 50. However, the one of the indicators, Body Pain (BP), was quite prevalent among most respondents (74%). This could be explained by the age of most participants, over 36 years old, which makes them more predisposed to have chronic diseases with pain syndrome. A systematic literature review by Figueiredo et al. (2022) depicted similar results where older people and females are the most vulnerable group who are predisposed to experience physical and mental impairments after recovery from COVID-19 [17]. Nevertheless, our study shows that the physical health was moderate-to-severe and higher among males than in women on all scales of the SF-36 questionnaire, while mental health was moderate-to-severe in both sexes.

Most of respondents expressed that they did not feel healthy after recovery from COVID-19 due to uncertainty of the pandemic and quarantine measures that have led to prolonged stays indoors. The lowest score in mental health was recorded among group of people who are younger than 30 years old. Both male and female had below average score of mental health. Similar study in the Philippines demonstrates that although individuals had better results in “Physical functioning” (PF) and “Role of limitation due to physical health” (RP) (85 % and 100%) their General and Mental health were poorer (50% and 44%) [18]. Other scholars show that after recovery from COVID-19 many individuals reported significant level of stress, anxiety, and depression symptoms, which might have long-term psychological consequences [19,20]. For example, in comprehensive cross-sectional research of over 1000 Chinese, scholars found 71.5%of participants expressed distress, 50.4% depression, 44.6% anxiety, and 34.0% insomnia, correspondingly [21]. In the retrospective perspective, O’Brien et al. (2022) claim that the issue of concentration and memorizing is keep growing in the period between six months to one year after recovery from COVID-19. Individuals complain about their lower ability to work and exercise at previous pace that affects their well-being [22]. This study did not focus on abilities of work and exercise after recovering from COVID-19, which should be looked at in the future studies.

The cumulative mean HRQoL score in the study was moderate-to-severe (45.89±10.37). Similar research conducted in China illustrated high HRQoL score (62.1±18. 8) [23]. Other studies show the total mean HRQoL score in patients with acute COVID -19 60.3 [24], and in patients with "Long COVID -19" - from 60.4 [24] to 86.4 [25], where a higher SF-36 score reflects better health status. The lowest HRQoL score (60.4) was in older patients (>65 years), and the highest HRQoL score (86.4) was among younger patients (54%, 18-46 years) and all patients without comorbidities. This indicates that the HRQoL of survey Kazakhstani population is relatively poorer than in other countries. Therefore, further qualitative research is warranted to explore individuals’ perceptions and experience of recovering physical and mental health in-depth.

Some limitations are present in the research. First, there was a high patient refusal rate; many patients failed to contact the research team or did not want to participate in the survey. Second, due to the absence of a specific mental status scale, only four aspects, namely SF, VT, MH, and RE, were assessed. Third, the presence or absence of symptoms characteristic of "protracted covid" was not traced, nor was the relationship to socioeconomic status, level of education, or presence of comorbidities. Moreover, due to the heterogeneity of results presentation on the effect of COVID-19 on HRQoL on the global scale and the lack of data that was captured one year after recovery from COVID-19, it was hard to compare the results of the study with results from other countries. However, compared to previous similar studies on HRQoL and Long COVID-19 that surveyed in the period between 4 and 12 weeks from the onset of symptoms, this study employed a survey a year after the inclusion of the last patient in the study, which gave a better opportunity to look at the long-term impairments after recovery from COVID-19.

Conclusion

Analysis of the HRQoL indicators of the study group in Nur-Sultan city, Kazakhstan showed that the male population had a better quality of life indicators on all scales of the SF-

36 questionnaire compared to the female population (p>0.05). Younger respondents under 30 had above-average scores on all scales except mental health. This score was the lowest among the other age groups. However, this age group rated overall health above average, regardless of gender. Respondents over 40 also rated their general health above the average level, and they were characterized by high scores on the scales of social activity and bodily pain. Mental health was rated below average among both sexes almost equally at 43.5(CI 95% 42; 45) among women and 43.7 (CI 95% 40.8; 46.6) among men (p=>0.05).

Thus, there is a need for additional research on quality of life and health status one year after COVID-19 using standard, more specific HRQoL instruments (e.g., EQ-5D, SF-6D) as well as disease-specific instruments with a standard method of HRQoL calculation and statistical presentation (i.e., mean scores for each dimension with standard deviation and 95% confidence interval, medians with range or patient shares). It would also be helpful to include additional questions on symptoms, comorbidities, and on detecting depression in COVID-19 patients.

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Molecular analysis of metallo-β-lactamase genes in some gram-negative bacteria and examination of the phylogenetic relationships of isolates

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Abstract

Aim: This study aimed to determine the susceptibility of carbapenem-resistant Gr (-) bacilli isolated from various clinical infections to various antibiotics and identify genes causing carbapenem resistance and their clonal relationships to elucidate the distribution of resistance in community and/or hospital-acquired strains.

Material and methods: In this study, antibiotic susceptibilities of 450 carbapenem-resistant Gr (-) bacilli isolated from clinical specimens at Cukurova University, Faculty of Medicine, Balcali Hospital, were investigated using phenotypic methods. The presence of carbapenems and □-lactamase genes were searched using polymerase chain reaction (PCR) and sequence analysis methods. Pulsed-field gel electrophoresis (PFGE) method was used to evaluate the phylogenetic relationship of the isolates.

Results: Based on the results, it was determined that 99.23% of the strains had gained resistance to meropenem, whereas 5.38% had developed resistance to colistin. The most dominant carbapenems genes in all isolates were OXA-51, OXA-23-like and OXA-24-like.

Conclusion: It was observed that the only antibiotic that could be used safely in carbapenem-resistant Gr (-) bacilli infections was colistin. In addition, when the clonal relationship of the strains was examined, it was found that the clones considered to be closely related persisted, and these clones settled in different clinics of our hospital.

Key words: Carbapenem, MBL, PFGE, CLSI

Introduction

Gr (-) bacteria are among the leading factors of hospital and community-acquired infections. Due to the outer membrane structure in the cell walls, it is resistant to many antibiotics compared to gram-positive bacteria, and it gains multiple resistance characteristics with the transfer of genetic material and/or the selective pressure of antibiotics in the hospital environment, creating problems for infections treatment caused by these bacteria. Resistant strains of these bacteria are more fatal when they cause infections, especially in patients who are observed in intensive care units. Enterobacteriaceae species and non-fermentative bacteria, such as *Acinetobacter and Pseudomonas,* are very important because they cause infections transmitted during hospital service and can easily transfer genes encoding resistance enzymes [1-3].

Enterobacteriaceae species, which are commensally found in flora, cause many infections as a primary or secondary pathogen in extraintestinal colonization, especially in people with diabetes, immunodeficiency, using immunosuppressive drugs, susceptible to infections, such as cancer patients, invasive instrument users and the elders. *Escherichia coli, Klebsiella spp.,*

Enterobacter spp., Proteus spp., Serratia spp., and Salmonella species are frequently isolated, especially in infections acquired during hospital service [4-7].

Acinetobacter spp and *P. aeruginosa species* are at the forefront among clinically important non-fermentative Gr (-) bacteria. Due to the increasing antibiotic resistance, there are challenges in the treatment of infections caused by these bacteria [8].

Many β-lactamase enzymes that can hydrolyze penicillins, cephalosporins, monobactams, and carbapenems have been found in the majority of Gr (-) bacteria. [9]. Metallo-β-lactamases (MBL) plays a critical role in developing resistance against carbapenem group antibiotics, which is a good option in treating severe infections caused by resistant bacteria against most antibiotic groups. Carbapenems are a type of β-lactamase that causes a broader spectrum of antibiotic resistance [10]. Carbapenems, also known as MBL, are quite common, especially among *E. coli* and *Klebsiella* strains. In hospitals, it is essential to know the type of β-lactamase in the causative pathogen to optimize the treatment protocols for the patients infected with Expanded Spectrum β-lactamase, induced β-lactamases and MBL-producing strains. This study aimed to enlighten the regional epidemiology of MBL positive isolates that cause major problems in treatment and to type the carbapenemase enzymes. Thus, the presence, types, and frequencies of MBLs in Gr (-) bacilli isolated from clinical samples of patients treated at Cukurova University, Balcali Hospital were investigated in this study. Phylogenetic analysis was performed to elucidate the distribution of the clonal association and the resistance of the community or the hospital-acquired strains.

Material and methods

A total of 450 Gr (-) bacilli isolates were isolated from various clinical materials at Cukurova University, Faculty of Medicine, Balcali Hospital and identified with the VITEK-II device, and were discussed to shed light on the epidemiology of nosocomial infections. Isolates were verified with conventional culture methods, biochemical tests, and the BD-Crystal Enteric/Nonfermented Identification kit. Phenotypic Carbapenems production was performed by Modified Hodge Test (MHT) according to Clinical Laboratory Standards Institute (CLSI) criteria. MHT is considered the gold standard for carbapenem resistance. *Escherichia coli* ATCC 25922 was selected as a reference strain. Imipenem-EDTA double-disk synergy test was used to detect MBL production in the isolates included in the study. To determine the susceptibility of the isolates included in the study against various antibiotics, the Kirby-Bauer Disk Diffusion test was performed according to CLSI recommendations [11]. Sequence analysis studies were conducted for the presence of carbapenemase and β-lactamase genes in isolates, using the automated system of "ABI Prism 310 DNA sequencer (Applied Biosystems)" with PCR-multiplex-PCR [12-14]. The data obtained by sequence analysis were compared with the gene bank database using the BLAST program on the "National Center for Biotechnology Information" (NCBI) web page (htpp: //www.ncbi.nlm.nih.gov/BLAST/). The pulsed-field gel electrophoresis method, which is the gold standard, was used to evaluate the phylogenetic relationship between isolates [15, 16]. This study was approved by the Non-Interventional Clinical Research Ethics Committee of the Medical Faculty of Cukurova University (Date: 06.12.2013 and Decision No: 19).

Results

In the current study, 450 isolates of MBL-resistant Gr (-) bacilli (*A. baumannii* (n=290), *P. aeruginosa* (n=75), *K. pneumoniae* (n=40), *E. coli* (n=20), *P. mirabilis* (n=10), *Enterobacter cloacae* (n=13) and *Chryseobacterium indologenes* (n=2)) were subjected to double-disk synergy and MHT tests; 130 of these isolates were found to be MBL positive. A. baumannii (n=88), P. aeruginosa (n=26), K. pneumoniae (n=9), E. coli (n=3), P. mirabilis (n=1), E. cloacae (n=1), and C. indologenes (n=2) were among the isolates. (Table 1).

Table 1 Species distribution of 130 isolates included in the study

Isolates	No	Percentage %
A. baumannii	88	67.69
P. aeruginosa	26	20
K. peumoniae	9	6.92
E. coli	3	2.30
C. indologenes	2	1.53
P. mirabilis	1	0.76
E. cloacae	1	0.76
Total	130	

Table 2 Antibiotic susceptibility test result of *A. baumannii* isolates

ANTIBIOTIC	RESISTANT		INTERMEDIATE		SUSCEPTIBLE	
	Number	Percentage %	Number	Percentage %	Number	Percentage %
AMIKACIN	51	57.95	3	3.4	34	38.63
AMPICILLIN SULBACTAM	85	96.59	3	3.40	-	-
CEFEPIME	13	97.72	1	2.27	-	-
CEFTAZIDIME	87	98.86	1	1.13	-	-
CIPROFLOXACIN	85	96.59	1	1.13	2	2.27
COLISTINE	1	1.13	-	-	87	98.86
GENTAMICIN	79	89.77	-	-	9	10.22
IMIPENEM	87	98.86	1	1.13	-	
LEVOFLOXACIN	77	87.5	11	12.5	-	
MEROPENEM	88	100	-	-	-	
PIPERACILLIN	88	100	-	-	-	
TETRACYCLINE	67	76.13	1	1.13	20	22.72
TIGECYCLINE	12	13.63	18	20.45	58	65.90
TRIMETOPRIM SULFAMETOXAZOL	82	93.18	-	-	6	6.81
PIPERACILLIN/TAZOBACTAM	88	100	-	-	-	

Kirby-Bauer Disk Diffusion test was performed according to CLSI recommendations to determine the sensitivity of 130 isolates included in the present study against various antibiotics. According to these results, more than 50% resistance was observed in *A. baummanii* isolates against other tested antibiotics except tigecycline and colistin antibiotics. Moreover, *Pseudomonas* isolates were more than 50% resistant to all tested antibiotics. In K. pneumoniae, high resistance rates were found against antibiotics other than colistin and amikacin. Also, in E. coli high resistance rates were found against antibiotics other than colistin and fosfomycin. The results of Kirby-Bauer Disk Diffusion antibiotic susceptibility tests are given in Tables 2-7. Considering all the isolates included in our study, the highest resistance developed against meropenem with 99.23%, and the lowest resistance against colistin with 5.38%.

Table 3 Antibiotic susceptibility test result of *P. aeruginosa* isolates

ANTIBIOTIC	RESISTANT		INTERMEDIATE		SUSCEPTIBLE	
	Number	Percentage %	Number	Percentage %	Number	Percentage %
AMIKACIN	19	73.07	-	-	7	26.92
AMPICILLIN SULBACTAM	26	100	-	-	-	-
CEFEPIME	16	61.53	7	26.92	3	38.46
CEFOPERAZONE SULBACTAM	18	69.23	7	26.92	1	3.84
CEFTAZIDIME	16	61.53	6	23.07	4	15.38
CIPROFLOXACIN	18	69.23	-	-	8	30.76
COLISTINE	-		-	-	26	100
GENTAMICIN	17	65.38	-	-	9	34.61
NETILMICIN	16	61.53	6	23.07	4	15.38
IMIPENEM	26	100	-		-	-
LEVOFLOXACIN	18	69.23	-	-	8-	30.76
MEROPENEM	25	96.15	1	3.84	-	-
PIPERACILLIN	2	76.92	6	23.07	-	-
TETRACYCLINE	26	100	-		-	-
TIGECYCLINE	25	96.15	1	3.84	-	-
TRIMETOPRIM SULFAMETOXAZOL	26	100	-	-	-	-
PIPERACILLIN/TAZOBACTAM	26-100	-	-	-	-	-

Table 4 Antibiotic susceptibility test result of *K. peumoniae* isolates

ANTIBIOTIC	RESISTANT		INTERMEDIATE		SUSCEPTIBLE	
	Number	Percentage %	Number	Percentage %	Number	Percentage %
AMIKACIN	4	44.44	5	55.55	-	-
AMPICILLIN	9	100	-	-	-	-
AMOXICILLIN-CLAVULANATE	9	100	-	-	-	-
CEFEPIME	8	88.88	1	11.11	-	-
CEFOXITINE	9	100	-	-	-	-
CIPROFLOXACIN	9	100	-	-	-	-
COLISTINE	3	33.33	-	-	6	66.66
ERTAPENEME	9	100	-	-	-	
GENTAMICIN	7	77.77	-	-	2	22.22
IMIPENEM	8	88.88	1	11.11	-	-
MEROPENEM	9	100	-	-	-	-
TRIMETOPRIM SULFAMETOXAZOL	8	88.88	-	-	1	11.11
PIPERACILLIN/TAZOBACTAM	9	100	-	-	-	-

Table 5 Antibiotic susceptibility test result of *E. coli* isolates

ANTIBIOTIC	RESISTANT		SUSCEPTIBLE	
	Number	Percentage %	Number	Percentage %
AMIKACIN	2	66.67	1	33.33
AMOXICILLIN-CLAVULANATE	3	100	-	-
AMPICILLIN	3	100	-	-
CEFOXIDINE	3	100	-	-
CEFTRIAXONE	3	100	-	-
CEFUROXIM	3	100	-	-
CYPROFLOXACIN	3	100	-	-
ERTAPENEM	3	100	-	-
FOSFOMYCIN	-	-	3	100
GENTAMICIN	2	66.67	1	33.33
IMIPENEM	3	100	-	-
MEROPENEM	3	100	-	-
NITROFURANTOIN	3	100	-	-
PIPERACILLIN/TAZOBACTAM	3	100	-	-
TRIMETHOPRIM/SULFAMETHOXAZOLE	3	100	-	-
COLISTIN	-	-	3	100

Table 6 Antibiotic susceptibility test result of *Chryseobacterium indologenes* isolates

ANTIBIOTIC	RESISTANT		SUSCEPTIBLE	
	Number	Percentage %	Number	Percentage %
AMIKACIN	2	100		
TICARCILLIN/CLAVULANIC ACID	2	100		
CEFTAZIDIME	2	100		
CEFOPERAZONE/SULBACTAM	2	100		
CIPROFLOXACIN	-	-	2	100
COLISTIN	2	100		
CEFAZOLIN	2	100		
ERTAPENEM	2	100		
CEFEPIME	2	100		
CEFOXITINE	2	100		
GENTAMICIN	2	100		
IMIPENEM	2	100		
LEVOFLOXACIN	-	-	2	100
MEROPENEM	2	100		
PIPERACILLIN-TAZOBACTAM	2	100		
TRIMETHOPRIM/SULFAMETHOXAZOLE	-	-	2	100
AZTREONAM	2	100		

Table 7 Antibiotic susceptibility test result of *Proteus mirabilis* and *Enterobacter cloacae* isolate

ANTIBIOTIC	Resistant (R)- Intermediate (I)- Susceptible (S)	
	Proteus mirabilis	*Enterobacter cloacae*
AMIKACIN	S	R
AMPICILLIN SULBACTAM	R	R
CEFEPIME	I	R
CEFOPERAZONE/SULBACTAM	R	R
CEFTAZIDIME	I	R
CIPROFLOXACIN	S	S
COLISTIN	R	S
GENTAMICIN	S	R
IMIPENEM	R	R
LEVOFLOXACIN	S	S
MEROPENEM	R	R
NETILMICIN	S	R
PIPERACILLIN	R	R
PIPERACILLIN-TAZOBACTAM	R	R
TETRACYCLINE	R	R
TIGECYCLINE	R	R
TRIMETHOPRIM/SULFAMETHOXAZOLE	R	R

Table 8 PCR result amplified β-lactamase genes in *A. baumanii* (88) isolates

β-lactamase genes	Number of isolates	Percentage%
OXA 51 LIKE	88	100
OXA 23 LIKE	54	61.36
OXA 24 LIKE	15	17.04
NDM-1	2	2.28
TEM	20	22.73
OXA 48 LIKE	3	3.40
GES	5	5.69
CTX-M	6	6.82
CTX-M 1	1	1.14
CTX-M 2	1	1.14
CTX-M 9	2	2.28

Table 9 β-lactamase genes amplified by PCR in *P. aeruginosa* (26) isolates

β-lactamase genes	Number	Percentage%
oxa 23 like	4	15.38
oxa 48 like	4	15.38
GES	2	7.70
CTX-M	1	3.85
VIM 1	1	3.85

Table 10 β-lactamase genes amplified by PCR in *K. pneumonia* (9) isolates.

β-laktamaz genleri	Number	Percentage%
oxa 48like	4	44.44
oxa 24 like	2	22.22
CTX-M	4	44.44
CTX-M 9	4	44.44

In-house PCR and Multiplex-PCR techniques targeting frequently seen β-lactamase genes were amplified in order to characterize carbapenemase genes.The most dominant carbapenems genes in all isolates had been found as OXA-51, OXA-23-like and OXA-24-like in Tables 8-11.

Sequence analyzes were performed by amplifying the detected MBL genes with specific primers. Also, it was defined at the sub-type of level. PCR results defining β-lactamase gene profiles within the species and the profiles of the gene sequenced are given in Table 12. Carbapenemase genes determined by sequence analysis were OXA-48, GES-11, VIM-1, NDM-1.

Other PCR analyses also revealed the presence of NDM-1 in one of three E. coli isolates, OXA-48 like genes in two isolates, and NDM-1, OXA-48 like genes, and CTX-M genes in one E. cloacae isolate. OXA-48, GES, and NDM-1 genes are given in Figure 1.

When the phylogenetic relationships of MBL resistant *A. baumannii* isolates (n: 88) were examined, it was determined that they were distributed in 17 clusters in a close relationship.

Table 11 Distribution of β-lactamase genes determined by multiplex-PCR in all isolates

Code	Species	β-lactamases	Code	Species	β-lactamases
1	*A. baumannii*	OXA-51-like + OXA-24-like	66	*A. baumannii*	OXA-51-like+OXA-23-like
2	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	67	*A. baumannii*	OXA-51-like+NDM-1
3	*A. baumannii*	OXA-51-like+OXA-24-like	68	*A. baumannii*	OXA-51-like+TEM+ OXA-23-like
4	*A. baumannii*	OXA-51-like+ OXA-24-like	69	*A. baumannii*	OXA-51-like+GES+ OXA-23-like
5	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	70	*A. baumannii*	OXA-51-like+ OXA-23-like
6	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	71	*A. baumannii*	OXA-51-like+ OXA-23-like
7	*A. baumannii*	OXA-51-like+ OXA-23-like+OXA-24-like	72	*A. baumannii*	OXA-51-like+CTX-M9
8	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	73	*A. baumannii*	OXA-51-like+ OXA-23-like
9	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	74	*A. baumannii*	OXA-51- like +TEM+ GES +OXA-23-like
10	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	75	*A. baumannii*	OXA-51-like+CTX-M+ OXA-23-like
11	*A. baumannii*	OXA-51-like+CTX-M2	76	*A. baumannii*	OXA-51-like+TEM+OXA-23-like+ OXA-48-like
12	*A. baumannii*	OXA-51-like+ OXA-24-like	77	*A. baumannii*	OXA-51-like+ OXA-23-like
13	*A. baumannii*	OXA-51-like	78	*A. baumannii*	OXA-51-like+ OXA-23-like
14	*A. baumannii*	OXA-51-like	79	*A. baumannii*	OXA-51-like+OXA-23-like
15	*A. baumannii*	OXA-51-like	80	*A. baumannii*	OXA-51-like+ OXA-23-like +OXA-24-like
16	*A. baumannii*	OXA-51-like+ OXA-23-like	81	*A. baumannii*	OXA-51-like+OXA-23-like+ OXA-24-like
17	*A. baumannii*	OXA-51-like	82	*A. baumannii*	OXA-51-like+ OXA-24-like
18	*A. baumannii*	OXA-51-like	83	*A. baumannii*	OXA-51-like
19	*A. baumannii*	OXA-51-like	84	*A. baumannii*	OXA-51-like+ CTX-M9+ OXA-23-like+ OXA-24-like
20	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-48-like	85	*A. baumannii*	OXA-51-like
21	*A. baumannii*	OXA-51-like	86	*A. baumannii*	OXA-51-like
22	*A. baumannii*	OXA-51-like	87	*A. baumannii*	OXA-51-like+GES+ OXA-23-like
23	*A. baumannii*	OXA-51-like	88	*A. baumannii*	OXA-51-like+CTX-M1
24	*A. baumannii*	OXA-51-like	89	*P. aeruginosa*	-
25	*A. baumannii*	OXA-51-like+GES	90	*P. aeruginosa*	-
26	*A. baumannii*	OXA-51-like+TEM+OXA-23-like	91	*P. aeruginosa*	OXA-23-like
27	*A. baumannii*	OXA-51-like+ TEM	92	*P. aeruginosa*	OXA-48-like
28	*A. baumannii*	OXA-51-like+ TEM+ OXA-23-like	93	*P. aeruginosa*	OXA-48-like
29	*A. baumannii*	OXA-51-like+ OXA-23-like	94	*P. aeruginosa*	-
30	*A. baumannii*	OXA-51-like+ TEM+ OXA-23-like	95	*P. aeruginosa*	VIM1
31	*A. baumannii*	OXA-51-like+ OXA-23-like	96	*P. aeruginosa*	-
32	*A. baumannii*	OXA-51-like	97	*P. aeruginosa*	GES
33	*A. baumannii*	OXA-51-like+ OXA-23-like	98	*P. aeruginosa*	-
34	*A. baumannii*	OXA-51-like	99	*P. aeruginosa*	-
35	*A. baumannii*	OXA-51-like+ TEM	100	*P. aeruginosa*	CTX-M
36	*A. baumannii*	OXA-51-like+ TEM	101	*P. aeruginosa*	OXA-48
37	*A. baumannii*	OXA-51-like+ OXA-23-like	102	*P. aeruginosa*	-
38	*A. baumannii*	OXA-51-like+ OXA-23-like	103	*P. aeruginosa*	OXA-48
39	*A. baumannii*	OXA-51-like+ OXA-23-like	104	*P. aeruginosa*	-
40	*A. baumannii*	OXA-51-like+TEM+OXA-23-like	105	*P. aeruginosa*	OXA-23
41	*A. baumannii*	OXA-51-like+NDM-1+ OXA-23-like	106	*P. aeruginosa*	-
42	*A. baumannii*	OXA-51-like	107	*P. aeruginosa*	-
43	*A. baumannii*	OXA-51-like+TEM+OXA-23-like	108	*P. aeruginosa*	OXA-23
44	*A. baumannii*	OXA-51-like+TEM+ OXA-23-like	109	*P. aeruginosa*	-
45	*A. baumannii*	OXA-51-like+ OXA-23-like	110	*P. aeruginosa*	-
46	*A. baumannii*	OXA-51-like+ OXA-23-like	111	*P. aeruginosa*	GES
47	*A. baumannii*	OXA-51-like+- OXA-23-like	112	*P. aeruginosa*	OXA-23
48	*A. baumannii*	OXA-51-like+ TEM	113	*P. aeruginosa*	-
49	*A. baumannii*	OXA-51-like	114	*P. aeruginosa*	-
50	*A. baumannii*	OXA-51-like	115	*K. pneumoniae*	OXA-48+ CTX-M9
51	*A. baumannii*	OXA-51-like+ OXA-23-like+ OXA-24-like	116	*K. pneumoniae*	OXA-48+ CTX-M9
52	*A. baumannii*	OXA-51-like+ OXA-23-like	117	*K. pneumoniae*	-
53	*A. baumannii*	OXA-51-like+OXA-23-like	118	*K. pneumoniae*	OXA-48
54	*A. baumannii*	OXA-51-like	119	*K. pneumoniae*	IMP
55	*A. baumannii*	OXA-51-like+ OXA-23-like	120	*K. pneumoniae*	-
56	*A. baumannii*	OXA-51-like+ TEM	121	*K. pneumoniae*	OXA-24+-CTX-M9
57	*A. baumannii*	OXA-51-like+ TEM+ OXA-23-like	122	*K. pneumoniae*	OXA-24+CTX-M9
58	*A. baumannii*	OXA-51-like+TEM+OXA-23-like	123	*K. pneumoniae*	OXA-48
59	*A. baumannii*	OXA-51-like+TEM+OXA-23-like	124	*E.coli*	OXA-48
60	*A. baumannii*	OXA-51-like+CTX-M+OXA-23-like	125	*E.coli*	NDM-1
61	*A. baumannii*	OXA-51-like+ OXA-23-like	126	*E.coli*	OXA-48
62	*A. baumannii*	OXA-51-like+TEM+OXA-23-like	127	*Chryseobacterium indologenes*	-
63	*A. baumannii*	OXA-51-like+ TEM+ OXA-23-like	128	*Chryseobacterium indologenes*	-
64	*A. baumannii*	OXA-51-like+GES+OXA-23-like	129	*Enterobacter cloacae*	NDM-1+OXA-48+CTX-M1
65	*A. baumannii*	OXA-51-like+ TEM+ OXA-23-like+ OXA-48-like	130	*Proteus mirabilis*	-

Table 12 Carbapenemase genes determined by sequence analysis

	OXA-48	GES	VIM	NDM
A. baumannii	3* OXA-48	5*GES-11	-	2*NDM-1
P. aeruginosa	4* OXA-48	1*GES-11+1*GES-12	1*VIM-1	-
K. pneumoniae	4* OXA-48	-	-	-
E.coli	2* OXA-48	-	-	1*NDM-1
Chryseobacterium indologenes	-	-	-	-
Enterobacter cloacae	1* OXA-48			1*NDM-1

Moreover, the strains that form the A1, A2, C1, D, F, H3, L1, N, O1, O2, P1, P2 and R clusters among these clusters were 100% similar. The largest cluster was formed by the L cluster with four sub-members (L1- 100%, L1-L2 96.8%, L2-L3 96%, L3-L-4 94%), and the second-largest cluster was the four sub-membered C cluster (C1-100%, C1-C2 95.8%, C2-C3 93.3%, C3-C4 87.9%). When we examined the distribution of β-lactamase genes in *A. baumannii* isolates, which we evaluated as closely related, OXA-51-OXA-24 genes in A cluster, OXA-51-OXA-23-OXA-24 genes in B, C, D clusters, OXA-51-TEM gene in cluster E, OXA-51-TEM-OXA23 genes in clusters F, G, H, I, J, OXA-51- OXA23 genes in clusters K, N, O, P, OXA-51-OXA-48- OXA-23 genes in cluster L, also OXA-51-GES-OXA-23 genes in cluster M. OXA-51 gene were detected in 88 *A. baumannii* isolates. OXA-23 gene was the second most common in closely related isolates. Later, TEM and OXA-24 genes were detected. In addition to this, *A. baumannii* isolates were persistent at Balcali Hospital and these isolates were settled in different clinics of the hospital (Figure 2).

When the phylogenetic relationships of *P. aeruginosa* isolates (n: 26) were evaluated, it was observed that they were distributed in six clusters (A-B-C-D-E-F) in close relation. It was analyzed that the C1 cluster was 100% similar and three sub-members (C1 100%, C1-C2 91.4%) constituted the largest cluster C. In addition, we determined that the members in the C cluster had the OXA-48 gene, the D cluster members had the GES gene, and the members in the E and F clusters had the OXA-23 β-lactamase gene (Figure 3).

As a result of the phylogenetic analysis of *K. pneumoniae* isolates (n: 9), we found that they were distributed into two closely related clusters (A-B). We determined that A1 and A2 clusters, which were sub-members of the four-member A cluster that made up the largest cluster, were 100% similar. We determined that the A1 cluster was closely related to the A2 cluster at a rate of 94.1% and the B1-B2 members that formed the B cluster were closely related to each other at the rate of 92.1%. We determined that the OXA-48-CTXM-9 genes in the A1 cluster, the OXA-24-CTXM-9 genes in the A2 cluster and the OXA-48 gene in the B cluster (Figure 4).

Figure 1 - Gel image showing the band profile of the 281 bp OXA-48 gene, 399 bp GES gene, 129 bp NDM-1 gene.

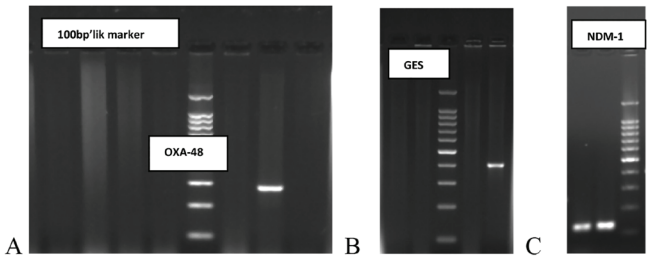


Figure 2 - Phylogenetic relationships of *A. baumannii* isolates.

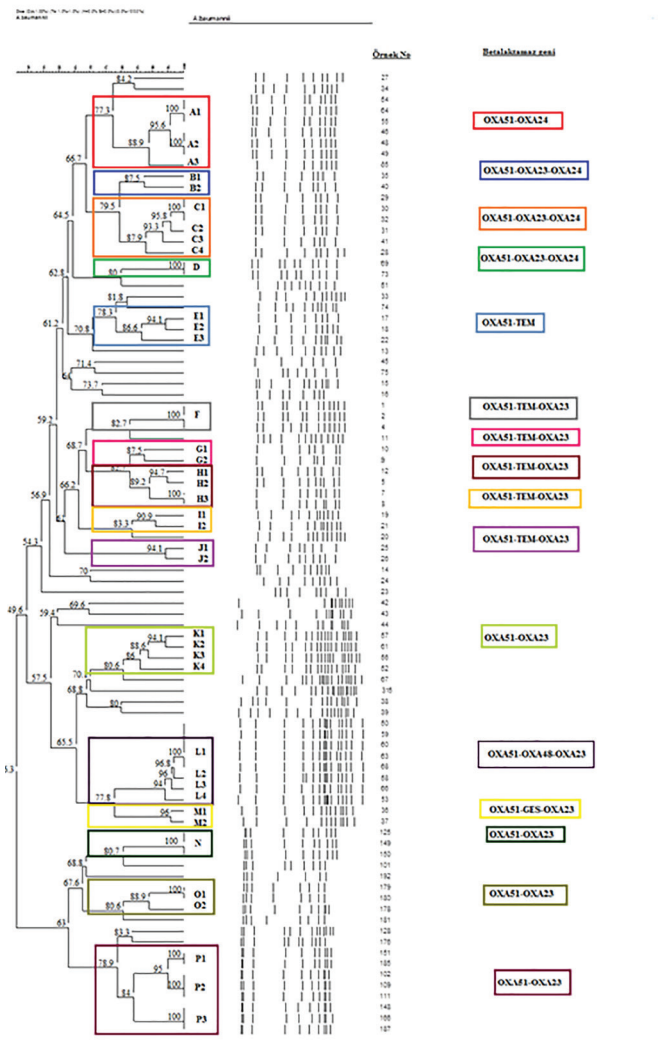


Figure 3 - Phylogenetic relationships of *P. aeruginosa* isolates

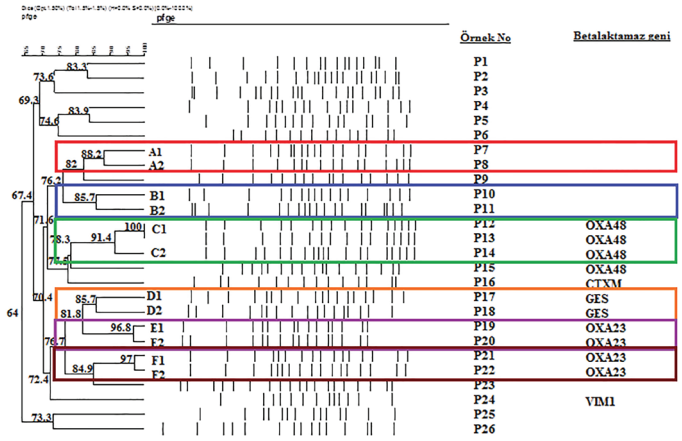
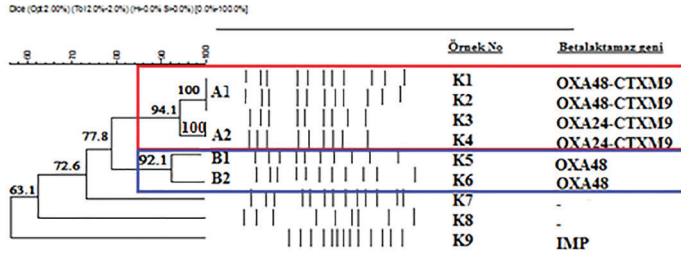


Figure 4 - Phylogenetic relationships of *K. pneumoniae*



Discussion

Bacteria showing resistance to antibiotics have become a major problem in the world and our country. Incorrect and unconscious use of antibiotics has led to an increase in multi-resistant Gr (-) bacilli. Resistance genes are studied with considerable interest by researchers worldwide.

In our study, as a result of phenotypic antibiotic susceptibility tests, we found that the isolates were 99.23% (129/130) resistant to meropenem, 98.46% (128/130) to imipenem, 88.46% (115/130) to ciprofloxacin, 86.92% (113/130) to cefepime and ampicillin- sulbactam, 70% (91/130) to tetracycline group antibiotics and 5.38% (7/130) to colistin. It is thought that there is a high degree of resistance against almost all antibiotics among non-fermenter bacillus and Enterobacteriaceae strains that have clinical importance in our region, and colistin can be used as the last option in untreatable infections.

After determining the antibiotic susceptibility of the isolates, carbapenemase genes were investigated genotypically. In *A. baumannii* isolates, OXA-51-like (100%), OXA-23-like (61.36%), TEM (22.73%), OXA-24-like (17.04%), CTX-M (6.82%), GES (5.69%), OXA-48-like (3.40%) and NDM-1 (2.28%) type β-lactamase genes were identified, respectively. In the study conducted by Ergin et al., they detected the genes OXA-23-like (31%), OXA-58-like (23%) and OXA-51-like [17]. When compared with our study, we found that OXA-51-like and OXA-23-like genes became more prevalent over the years. Another finding in our study was the detection of GES-11 type A group carbapenemase in five isolates (5.69%). The first isolate producing GES enzyme in our country was found by Bogaerts et al. in a patient transferred to Belgium in 2010 [18]. Later in 2013, it was reported that the GES enzyme was synthesized by *A. baumannii* isolates in two different studies conducted by the groups of Cicek and Zeka [19,20]. The high resistance rates in *A. baumannii* isolates in our study can be interpreted due to either carbapenemase genes or combined β-lactamases. However, future studies on porin protein permeability and efflux systems are important in terms of elucidating these mechanisms.

In our study, OXA-23-like, OXA-48, GES-11, GES-12, CTX-M and VIM-1 β-lactamase genes were detected in P. aeruginosa isolates, respectively. In their review of the literature, Breidenstein et al. attributed the reason why *P. aeruginosa* is much more resistant to antimicrobials than other Gr (-) bacilli due to its reduced outer membrane permeability, improved efflux systems, different porin numbers and structures from other bacteria, and various resistance mechanisms, such as these [21]. Fernandez et al. reported that it could adapt better than other bacteria against various environmental stress factors, such as antibiotic pressure, nutrient deficiency, and insufficient breeding environment [22]. Such changes are controlled by genes, such as *crc, lon, psrA, ampD, gyrA, nalA, nfxB, mexZ, phoQ* in the genome [21]. In line with this information, the fact that β-lactamase genes were detected in *P. aeruginosa* isolates was because carbapenem resistance occurred with efflux systems or membrane permeability modifications.

We identified OXA-48 (44.44%), OXA-24 (22.22%), CTX-M (44.44%) and CTX-M9 genes in *K. pneumoniae* isolates included in this study, respectively. Guran et al., in a study they conducted in 2011, determined the frequency of CTX-M genes in community-acquired *K. pneumoniae* isolates as 88.8% [23]. However, in a similar study conducted in 2014, they found the total CTX-M frequency among Carbapenem-resistant *K. pneumoniae* isolates as 52% (13/25) [11]. In this study, the frequency of CTX-M was 44.44%. The most common β-lactamase genes in *K. pneumoniae* isolates in our study were OXA-48 and CTX-M genes, with a rate of 44.44%. Alp et al. reported in their epidemiological study that the prevalence of the OXA-48 gene in *K. pneumoniae* isolates was 91.5% in 2013 [24]. Nazik et al. reported that the prevalence of the OXA-48 gene in K. pneumoniae isolates was common in their study between 2011-2012 [25, 26]. In our study, we detected NDM-1 in one of the 3 *E.coli* isolates and OXA-48-like genes in 2. OXA-48 enzyme in *K. pneumoniae* isolates was identified for the first time in 2001 in Turkey. In later years, it was detected in various Enterobacteriaceae species and *E. coli* [27, 28]. Turkey is a country that is now considered to be endemic to the OXA-48 enzyme. It is stated that the strains carrying this enzyme are now circulating in society [29].

Carbapenemase enzyme types differ between regions or countries depending on the preferred frequency of antibiotic use. The movements of the strains producing carbapenemase enzyme should be limited with the help of molecular epidemiological methods in the community and hospital also the development of new resistance should be minimized. In molecular epidemiological studies conducted in many countries, especially in developing countries. It was observed that there was a large increase in the prevalence of strains producing carbapenemase enzyme in Gr (-) bacilli isolated from both hospital and community-acquired infections.

In the study conducted by Yang et al. in Korea, it was determined that all the carbapenem-resistant *A. baumannii* strains were clonally related to PFGE method [30]. In a study conducted in Greece, Pournaras et al. analyzed 17 carbapenem-resistant *A. baumannii* isolates using the PFGE method and identified six different clones [31]. In the 2009 SENRTY surveillance study covering ten countries (China, India, Indonesia, Thailand, Korea, Taiwan, Singapore, Australia, Hong Kong, and the Philippines), the results of the PFGE analysis of the Carbapenem-resistant *A. baumannii* isolates carrying the OXA-23 gene were determined and it was emphasized that it had an epidemic potential for these countries [32]. In a study conducted in the USA, it was stated that Carbapenem-resistant *A. baumannii* isolates carrying the OXA-23 gene were a significant threat for hospital infections [33]. Vahapoğlu et al. stated in their study that the genes in carbapenem-resistant *A. baumannii* isolates were plasmid-derived and showed multiple clonalities with PFGE [34]. In a study conducted in South Korea, it was determined that there was 85% and more clonal association in 35 *P. aeruginosa* isolates with the IMP-6 gene using the PFGE method. It was observed that a single isolate spread throughout the country [35]. Between 2002 and 2006, Pitout et al. investigated MBL production and the presence of clonal association in 528 carpaneme-resistant *P. aeruginosa* isolates, 518 of which were isolated from four different hospitals and 10 from environmental samples. They detected VIM-2 in 178 isolates and IMP-7 in seven isolates. 178 VIM-positive isolates showed a binary closely related pattern. One hundred fifty-four of them formed a group, while 21 formed a different group. They found that 3-VIM-positive isolates were unrelated to these patterns. They reported that the IMP positive isolates were in a different group from these groups [36].

In our study, which was conducted to monitor the prevalence and movements of the strains producing hospital and community-acquired carbapenemase enzyme, it was observed that *A. baumannii* isolates (n: 88) were distributed in 17 clusters (A1, A2, C1, D, F, H3, L1, N, O1, O2, P1, P2, R) closely related, the strains forming these clusters were 100% similar. The most

distributed β-lactamase genes were OXA-51, OXA-23, TEM, OXA-24. It was detected that *P. aeruginosa* isolates (n: 26) were distributed in six clusters (A-B-C-D-E-F) closely related, C1 cluster was 100% similar, members in cluster C had OXA-48, E and F cluster members had OXA-23 β-lactamase genes. *K. pneumoniae* isolates (n: 9) were distributed in two closely related clusters (A-B). It was determined that the sub-member A1-A2 cluster of the four-member A cluster forming the largest cluster was 100% similar and the closely related *K. pneumoniae* isolates had OXA48-CTXM9 β-lactamase genes. In our study, similar to other studies, it was observed that some clones that we evaluated in close relation with the PFGE method persist in our hospital and these clones were located in different clinics. In our study, it was determined that there was a high degree of resistance against almost all antibiotics among non-fermenter bacillus and Enterobacteriaceae strains, which were clinically important in our region as in the whole world. An increase in the prevalence of strains with carbapenemase activity was found. In addition, it was observed that strains synthesizing NDM-1 type carbapenemase, which are very difficult to treat, became widespread in our country.

As a result, considering the data of epidemiological surveillance studies in our region, it is necessary to take urgent measures to develop new and more effective control measures, review and revise existing control measures, and develop and implement more rational, disciplined strategies. More regional epidemiological studies are needed in our country to restrict the motility of strains carrying antibiotic resistance genes.

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Investigation of LncRNAs expression in patients with hepatitis B virus

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Abstract

Aim: Patients infected with the hepatitis B virus (HBV) are at a higher risk of cirrhosis and hepatocellular carcinoma. Despite the recent advancement of antiviral therapy, many patients still cannot respond to existing therapies. Hence, to detect the changes in liver function earlier, non-invasive methods are needed. Long non-coding RNAs (lncRNAs) play important roles in essential biological process as well as human cancer. LncRNAs may be used as biomarkers in human diseases. Thus, in this study, we purposed to analyze the expression levels of lncRNAs (HOX transcript antisense RNA (HOTAIR), maternally expressed 3 (MEG-3), highly upregulated in liver cancer (HULC)) in patients with hepatitis B virus and healthy volunteers.

Methods: We selected three lncRNAs as candidate lncRNAs based on their association with liver disease. Whole blood samples were collected from 40 patients with HBV and 48 healthy volunteers. The expression levels of all the samples were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). Statistical analysis was implemented using GraphPad Prism software. A p-value lower than 0.05 was statistically meaningful.

Results: The expression levels of HOTAIR and HULC were remarkably upregulated in the plasma of the patients with HBV compared with healthy control ($p<0.05$). In contrast, no significant difference in MEG-3 expression levels was observed between groups.

Conclusion: Our findings showed that the expression of HOTAIR and HULC in plasma might be new promising diagnostic and/or prognostic biomarkers for HBV.

Keywords: Long non-coding RNA, Hepatitis B Virus, HOTAIR, HULC

Introduction

Hepatitis B is common worldwide and is a severe health problem. Approximately 257 billion people have a past or present hepatitis B virus (HBV) infection, and chronic HBV carrier is more than 350 million [1]. In patients infected with HBV, it has been reported that there is a risk of death from cirrhosis, liver failure, hepatocellular cancer (HCC) and HBV-related liver disease, and this group constitutes 15-40% of patients with HBV-infected [2]. More than 887,000 people die yearly because of chronic and acute HBV-related diseases [3]. According to hepatitis B surface antigen (HBsAg) positivity in Türkiye, the prevalence of HBV is between 2-8%, and it is in the middle endemicity region [4].

Despite the improvement of antiviral therapy, many patients still cannot respond to novel treatments. Thus, the determination of non-invasive methods to detect changes in liver functions is urgently required to improve the clinical outcomes of HBV infection [5].

Non-coding RNAs (ncRNAs) are regulators of complex biological processes, and their dysregulation plays a role in the pathogenesis of many diseases, including HBV [6]. The ncRNAs involved in gene regulation are basically classified into two classes based on their length. Long non-coding RNAs (lncRNAs) are single-stranded RNA molecules that are not translated into proteins at least 200 nucleotides long [7]. LncRNAs play important roles in the essential pathophysiological processes including apoptosis, inflammation, cell cycle, differentiation, and proliferation by modulating gene expressions of their target at the transcriptional and translational level. Further, lncRNAs act as strong regulators for metabolic activity by modifying protein molecules [8-10,13]. Moreover, the lncRNAs take part in

antiviral defense and regulates cell development in the immune system. Mutation or abnormal expression (up-down regulation) of lncRNAs is closely associated with human pathologies. Expression of cellular lncRNAs can change in response to viral replication or viral protein expression. lncRNA functions as negative or positive regulators of the antiviral response in the innate immune response. It regulates the cellular activities (macrophages, NK cells) involved in the innate immune response [11, 12, 13, 14]. Recently, many studies have shown that lncRNAs, such as HOX Transcript Antisense Intergenic RNA (HOTAIR), Highly upregulated in liver cancer (HULC), and maternally expressed gene 3 (MEG-3), have a role in the development of HBV-associated HCC [9, 20]. HBV-mediated interference with HOTAIR represents a crucial mechanism by which the virus can promote tumor development by deregulating fundamental metabolic and cell cycle regulatory processes that are needed to maintain the hepatocytes highly differentiated. HULC has been implicated in lipogenesis and angiogenesis in hepatoma cell lines, and siRNA knockdown of HULC has been shown to deregulate proliferation-related genes. Furthermore, some studies have reported that the expression of HOTAIR and HULC were upregulated in inflammatory processes and their upregulation inhibits proliferation and promotes apoptosis and inflammatory responses [6, 24]. Based on the aforementioned studies, in our study, we aim to investigate the expression levels of HULC, HOTAIR and MEG-3 in peripheral blood samples of patients with HBV.

Material and methods

Patient and control groups

Forty patients were randomly selected (21F, 19M) with untreated chronic patients with HBV who had been applied to the Mustafa Kemal University, Health Practice and Research Hospital, the Department of Gastroenterology. Patients under 18 years old, pregnant women, active infection, chronic diseases (DM, COPD, CRF, CHF), cirrhotic patients and malignancy were excluded from this study. The control group consisted of healthy volunteers (25 F, 23 M) compatible with age and gender.

In our study, demographic and clinical features of HBV and control groups were considered age, gender, HbsAg (Hepatitis B surface antigen), Anti-HBs (Antibody for HBsAg), Anti-HBe (Antibody for HBeAg), HBeAg (Hepatitis B envelope antigen), Albumin, ALT (Alanine aminotransferase), and AST (Aspartate aminotransferase), TBil (Total bilirubin), DBil (Direct bilirubin), AFP (Alpha-fetoprotein), Hb (Hemoglobin), Plt (Platelets), and PT/INR (Prothrombin Time and International Normalized Ratio).

Ethical approval

An informed consent form was obtained from all the participants who participated in this study. This study was approved by the Clinical Ethics Committee of Hatay Mustafa Kemal University (ethical approval number 2016/63). Further, the present study was conducted according to the Declaration of Helsinki.

Sample collection and gene expression analyses

Peripheral blood samples from patients and healthy controls at diagnosis were collected. Peripheral blood samples were transferred into a tube containing EDTA, and all tubes were centrifuged at 3000 rpm, + 4 °C for 15 minutes and then tubes were stored at – 70 ºC for further gene expression analyses.

Measurement of lncRNA levels by Real-time PCR

Total RNA isolation from plasma was performed using an extraction kit (QIAGEN RNeasy Mini Kit Cat No: 74104), and total RNA quality was determined by A260/A280 ratio and 1.5 agarose gel electrophoresis. Subsequently, total RNA was converted into complementary DNA (cDNA) using the cDNA synthesis kit (QIAGEN, RT2 HT first strand kit) according to the manufacturer's instructions. The expression levels of lncRNAs (HOTAIR, HULC and MEG-3) were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR) using the Rotor-Gene 600 device (Corbett Research, Australia). Specific amplification was confirmed by melting curve analysis and The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of lncRNAs.

Statistical analysis

Using https://www.qiagen.com/tr/shop/genes-and-pathways/data-analysis-center-overview-page/?akamai-feo=off site, the results were uploaded to the analysis system as an excel table. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of lncRNAs. GraphPad Prism 6.0 (GraphPad Software) was used for statistical analyses and plotting the graphs. A p-value lower than 0.05 was accepted statistically significant.

Results

Plasma samples were taken from 48 healthy controls. (23 males and 25 females,), 40 patients with HBV (19 males and 21 females). No significant difference was observed in age and sex ratio among the two groups ($p>0.05$).

Analyses of lncRNAs expression in patients with chronic HBV infection

In 40 patients with chronic HBV infection, lncRNA expression was 2-fold higher than the control group during initial diagnosis ($p<0.05$). In other words, our results showed that the expression levels of HOTAIR and HULC were notably up-regulated in the plasma of patients with HBV compared with healthy control and LncRNAs exhibited more than two-fold increased ($p<0.05$). In contrast, we did not observe any significant difference in MEG-3 expression levels between the groups (Figures 1, 2, 3).

Other biochemical parameters were analyzed using routine techniques, and our data showed that the levels of some parameters, such as AST, ALT, TBA, Crea, UA, GGT, and ALB were significantly different between healthy controls and patients with HBV ($p<0.001$). Further, no significant difference was determined for the level of TP and TBIL among two groups ($p>0.05$) (Table 1).

Discussion

ncRNAs play a major role in the detection of immunomodulators involved in the host's resistance to hepatitis viruses and determine the prognosis. Moreover, there is a need to identify novel non-invasive biomarkers that allow early detection of changes in liver functions.

Some studies have shown that lncRNAs might take part in many different biological processes, such as cell proliferation, differentiation, cell cycle, apoptosis, and invasion. It has also been shown that lncRNAs can play an essential role in the regulation of the eukaryotic genome [12-15].

Figure 1 - Comparison of expressions of HOTAIR, HULC and MGE-3 in healthy control and patients with HBV.

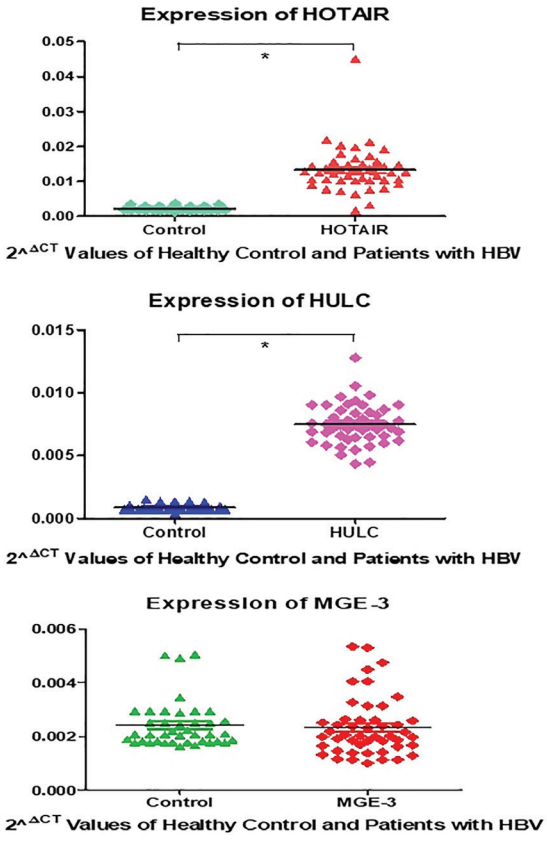


Figure 2 - Magnitude of gene expression

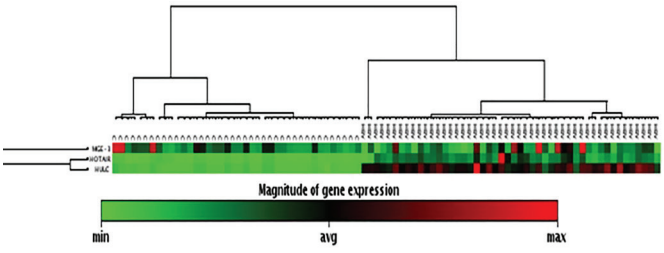
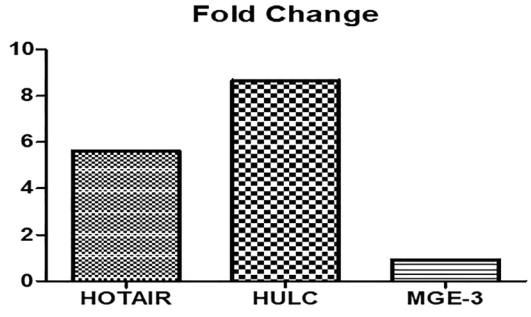


Figure 3 - The relative expression levels of HOTAIR (a), HULC (b) and MGE-3 (c) in healthy control and patients with HBV



Especially, dysregulation expression of lncRNAs has been associated with many diseases in humans, including cancer [16-17].

In response to the viral replication or viral protein expression, the expression of cellular lncRNAs can alter, whereas many cellular lncRNAs make a response to antiviral pathways induced by infection. Indeed, many lncRNAs can act as the innate antiviral response's positive or negative regulators. Our current knowledge of the identities and functions of lncRNAs in infected cells is very limited. However, identifying new cellular pathways has already been assisted by new research in this area and that could help for the improvement of therapeutic tools in the treatment of viral infections, autoimmune diseases, neurological diseases, and cancer [18].

Table 1 Demographic and clinical features of HBV and control groups

Parameter	Chronic Patients with HBV	Healthy Control	*p*-value
Age Median (range)	±35	±34	$p>0.05$
Gender	19 F 21 M	25 F 23 M	$p>0.05$
AST Median (range)	32.00	21.00	$p<0.001$
ALT Median (range)	30.00	17.00	$p<0.001$
TBA	3.20±2.42	2.8±2.32	$p<0.001$
Crea	35.40±31.02	62.12±12.4	$p<0.001$
GGT	35.40±31.02	18.06±6.84	$p<0.001$
ALB	45.60±2.42	43.21±2.05	$p<0.001$
TP Median (range)	1.07	1.03	$p>0.05$
TBIL Median (range)	0.60	0.60	$p>0.05$

HOTAIR is a kind of lncRNA associated with human HOX loci and contributes to metastasis in HCC [15]. Functional analyses indicated that HOTAIR supported HBV transcription and replication by elevating the activities of HBV promoters. In our study, we investigated the clinical importance of HOTAIR in patients with HBV and detected that HOTAIR expression is upregulated in patients with HBV, which is consistent with previous studies. A recent study revealed that HOTAIR expressions were increased in patients with chronic hepatitis B and its elevated levels are relationship with liver biomarkers [6]. In another study, *Zhong et al.* reported that HOTAIR expression is increased in patients with HBV compared to the control group [21]. Furthermore, these results highlight that the upregulation of HOTAIR plays a crucial role in the inflammatory process. [6].

HULC is another lncRNA which is highly expressed in liver cancer and plays a significant role in liver carcinogenesis [19-20]. *Panzitt et al*. reported that HULC was extremely tissue specific [20]. *Zhong et al.* found that expression of HULC is elevated in patients with HBV compared to the control group [21]. Moreover, *Wang et al.* showed that the expression of HULC was upregulated in inflammatory processes [25]. In our study, we found that HULC expression was upregulated in patients with chronic hepatitis B compared to the control group, which is consistent with previous studies.

MEG-3 is located on human chromosome 14q32.2 and is widely expressed in normal tissues [22]. It was reported that MEG-3 is down-regulated in cancerous tissues [23]. In addition, *Chen et al.* unveiled that MEG-3 is lowly expressed in patients with chronic hepatitis B [24]. In contrast, we did not observe a significant difference in MEG-3 expressions between healthy controls and patients with HBV.

In favour of its capability to enable HBV transcription and replication, HOTAIR and HULC may function as new HBV diagnostic and therapeutic biomarkers. Therefore, elevated lncRNA levels in patients on antiviral therapy may be associated with HCC. In addition, we thought that the altered levels of gene expression in patients receiving antiviral treatment might be interesting because the current treatment may prevent the development of HCC through the regulation of gene expressions, and it is a subject worth investigating. Therefore, in our following study, we plan to examine lncRNA expression levels in patients with HBV receiving antiviral therapy.

In brief, lncRNAs play an essential role in the regulation of gene expression and are involved in various biological processes, including inflammatory response and apoptosis. Thus, it is not surprising that altered lncRNA expression is linked to a variety of human diseases, including diabetes, infection, autoimmune diseases, and most notably, cancer. In this regard, growing evidence suggests that lncRNAs may play a role in the pathogenesis of various cancers, including gastric, colon, lung, and pancreatic cancers, as well as glioma, melanoma, and hepatocellular carcinoma (HCC). It can be used as a biomarker in at least some patients with HBV and HBV-associated HCC, especially in connection with other risk factors, such as age, alcohol, smoking, obesity, and metabolic syndrome. However, we should note that we need advanced studies for this.

Limitations

There are some limitations in our study. Our study included a small sample group. Further research with larger samples and patients with HBV-related HCC can validate the findings and produce more reliable results. Possible confounding factors of our study may be that the participants did not have similar characteristics (e.g., age, gender, other chronic diseases). Despite these limitations, the research described above provides useful information for further screening circulating lncRNAs that may serve as HBV biomarkers.

Conclusion

As a result, the plasma level of HULC and HOTAIR in patients with HBV could be used as a biomarker for disease progression. Further research is needed to verify the prognostic role of HULC and HOTAIR in patients with HBV and to better understand the relationship between candidate lncRNAs and the hepatitis B virus infection.

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Prediction of pulmonary complications following spine surgery: The ASA and ARISCAT risk indexes

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Abstract

Objective: We aimed to evaluate the effectiveness of predicting postoperative pulmonary complications (PPCs) following spine surgery, comparing American Society of Anesthesiologist (ASA) and Assess Respiratory Risk in Surgical Patients in Catalonia (ARISCAT) risk scoring systems.

Material and methods: We reviewed 377 patients aged≥18 years who had undergone vertebral surgery. Demographic data, comorbidities, ASA classification, body mass index, ARISCAT risk score, pulmonary complications developing with in the postoperative 1st month were assessed.

Results: A total of 377 patients, 221 (58.6%) women and 156 (41.4%) men, mean age of 59±11.8 years were evaluated. Out of the 377 patients, 73 (19.4%) patients were ASA I, 235 (62.3%) patients were ASA II, 69 (18.3%) patients were ASA III, and the mean ARISCAT score was 22.51±8.38. In the postoperative period, PPC was identified in 30 (8%) patients, with atelectasis in 15 (4%), pneumothorax in 4 (1.1%), pneumonia in 4 (1.1%), respiratory failure in 4 (1.1%), bronchospasm in 2 (0.5%) patients, and pulmonary embolism in 1 (0.3%) patient. There was a statistically significant correlation between the presence of PPC and ASA score, and between the presence of PPC and the ARISCAT levels (p=0.000, p=0.000). The incidence of PPC increased with increasing ASA scores. The ARISCAT scores were higher in patients who developed PPC. The hospital stay of patients with PPCs were longer than other patients (p=0.000).

Conclusion: In our study, in which ASA classification and ARISCAT risk index were compared as a means to predict PPC, both scores were found to be effective.

Key words: ASA, postoperative complications, spine, surgery

Introduction

Postoperative pulmonary complications (PPCs) are postoperative complications occurring in 2–70% of patients, which are associated with increased postoperative morbidity, mortality, and prolonged hospital stays, resulting in increased patient care costs. PPCs are important determinants of 30-day mortality [1,2].

Common PPCs include atelectasis, bronchospasm, pneumonia, pleural effusion, pulmonary edema, hypoxemia, and respiratory failure. The patient's general health status is an important determinant of pulmonary risk [3]. The American Society of Anesthesiologists (ASA) physical status classification is a classification system used by anesthesiologists and surgeons to describe overall health status of the patient. The ASA is used to estimate patients' perioperative risk. Patients are classified according to functional limitations created by existing medical problems. A high ASA score is associated with increased functional dependence, increased risk of complications, need for postoperative intensive care unit (ICU), prolonged hospital stays, and increased mortality [4]. The Assess Respiratory Risk in Surgical Patients in Catalonia (ARISCAT) risk index is a classification system used to predict PPCs. The ARISCAT risk index is calculated by assessing the patient's history, physical examination, monitoring, laboratory results, and information about the surgical procedure performed [5].

Due to degenerative vertebral pathologies such as spinal stenosis and degenerative spondylolisthesis that have been increasing with the increase in the elderly population, spine surgery has become one of the frequently performed surgeries worldwide [6]. Despite technological developments and modern surgical techniques, PPCs are common after spine surgery due to conditions such as prolonged surgery, large elderly patient population, presence of comorbidities, underlying pulmonary diseases, smoking, and blood transfusion. Given the incidence of postoperative pulmonary complications, risk estimation should be prioritized and be a standard element of preoperative medical assessments. The present study hypothesized that both the ARISCAT risk index and ASA score would be predictors of pulmonary complications following spine surgery. The aim of our study is to compare the efficacy of the ASA classification and the ARISCAT risk index in predicting PPCs following posterior spine surgery.

Material and methods

Patient population

The study was conducted in accordance with the Declaration of Helsinki. Following the approval of the study protocol by the Local Ethics Committee, patients who underwent spine surgery in our hospital between January 2019 and December 2020 were included in the study. The data of the patients were reviewed retrospectively using the hospital information system and archive records. Patients aged ≥18 years, who underwent posterior spine surgery were included in the study, and patients whose necessary medical information could not be accessed due to insufficient medical records were excluded. Patients' demographic data, comorbidities, ASA classification, body mass index (BMI), ARISCAT risk score (age, preoperative oxygen saturation and hemoglobin levels, type of incision, length of surgery, history of lower respiratory tract infection within one month prior to the surgery and emergency surgery, pulmonary complications within the postoperative one month and the time of complication development were recorded. Pulmonary complications such as atelectasis, lower respiratory tract infection, pneumonia, pulmonary embolism, pneumothorax, bronchospasm, and respiratory failure within the postoperative one month were examined. Postoperative pulmonary complications were determined by reviewing the hospital information system and patient medical records as well as by conducting patient phone interviews.

Assessment of postoperative pulmonary risk

The ASA scores that were determined by an anesthesiologist via preoperative patient assessment were recorded (Table 1). The ARISCAT risk indexes of the patients were calculated using age, preoperative oxygen saturation and hemoglobin levels, type of incision, length of surgery, history of lower respiratory tract infection one month before the surgery, and emergency surgery, which were retrieved from medical records of the patients (Table 2).

Table 1 The American Society of Anesthesiologists physical status classification system

ASA I: A normal healthy patient
ASA II: A patient with mild systemic disease
ASA III: A patient with severe systemic disease
ASA IV: A patient with severe systemic disease that is a constant threat to life
ASA V: A moribund patient who is not expected to survive without the operation
ASA VI: A declared brain-dead patient whose organs are being removed for donor purposes

Table 2 The assess respiratory risk in surgical patients in Catalonia Risk Index: Independent predictors of postoperative pulmonary complications

Risk Factor	Risk score
Age, years	
≤50	**0**
51-80	**3**
>80	**16**
Preoperative O2 saturation	
≥ 96%	**0**
91%-95%	**8**
≤ 90%	**24**
Respiratory infection in the last month	**17**
Preoperative anemia	
Hemoglobin >10 g/dL	**0**
Hemoglobin ≤ 10 g/dL	**11**
Surgical incision	
Peripheral	**0**
Upper abdominal	**15**
Intrathoracic	**24**
Duration of surgery	
≤ 2 hours	**0**
2-3 hours	**16**
> 3 hours	**23**
Emergency surgery	**8**
Risk class, No. of points in risk score (pulmonary complication rate)	
Low < 26 points	
Intermediate 26-44 points	
High > 44 points	

Definitions of PPC

Cough, sputum, fever (≥38°C) and leukocytosis, in addition to these symptoms, the presence of new infiltrates on chest X-ray was defined as pneumonia [7]. Suspicion of pulmonary embolism was confirmed by clinical and laboratory data (D-dimer, chest radiographs, arterial blood gases, and computed tomography. Atelectasis was evaluated as a decrease in breath sounds on physical examination and a shift of the mediastinum and hemidiaphragm towards the atelectatic area on chest X-ray in addition to the clinical picture. Pneumothorax lung air in the pleural space without a vascular bed on the X-ray, bronchospasm was evaluated as a newly detected wheezing and response to treatment with a bronchodilator. Respiratory failure was defined as oxyhemoglobin saturation measured by pulse oximetry <90%, PaO2<60 mmHg in arterial blood gas, and PaO2:FiO2 ratio < It was defined as 300 mmHg [8].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences IBM SPSS 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). The Shapiro-Wilk test was performed to determine that the data is normally distributed. Descriptive statistics were expressed as mean and standard deviation or median (minimum–maximum) for quantitative

data, and frequency and percentage for qualitative data. The Mann-Whitney U test was used for the non-normally distributed variables. The Pearson's Chi-square, Fisher-Freeman-Halton and Fisher's Exact Chi-square tests were used to analyze categorical data. In case of significance, the Bonferroni test, one of the multiple comparison tests, was used. The Spearman's correlation coefficients were used to analyze the relationships among variables. The significance level was set at p=0.05.

Results

The study group consisted of 400 patients out of which 23 patients were excluded due to insufficient patient medical records. A total of 377 patients, 221 (58.6%) females and 156 (41.4%) males, with a mean age of 59±11.8 (18-86) years, were assessed. Demographic data of the patients are presented in Table-3. The ASA score was ASA I in 73 (19.4%) patients, ASA II in 235 (62.3%) patients, ASA III in 69 (18.3%) patients. The mean ARISCAT score was 22.51±8.38 (3–62).

Table 3 Demografic data and comorbidities

Gender (n, %)	
Male	156 (41.4%)
Female	221 (58.6%)
Age (years) (mean±sd)	59±11.8
Smoking History (n, %)	
Current	87 (23.1%)
Never	271 (71.9%)
Prior	19 (5%)
History of previous surgery (n, %)	
Yes	258 (68.4%)
No	119 (31.6%)
Diabetes Mellitus (n, %)	111 (29.4%)
Hypertension (n, %)	149 (39.5%)
Coronary Artery Disease (n, %)	40 (10.6%)
Asthma (n, %)	15 (4%)
COPD (n, %)	15 (4%)
Congestive Heart Failure (n, %)	6 (1.6%)
Cardiac Rythm Disorder (n, %)	8 (2.1%)
BMI (kg/m2) (n, %)	
<25 (Normal)	88 (23.3%)
≥25 & <30 (Overweight)	187 (49.6%)
≥30 & <35 (Obese)	76 (20.2%)
≥35 (Severely Obese)	26 (6.9%)

ASA: American Society of Anesthesiologist; COPD: Chronic Obstructive Pulmonary Disease
BMI: Body mass index

The surgery was elective in 348 (92.3%) patients and was performed under emergency conditions in 29 (7.7%) patients. Spine surgery was performed due to degenerative vertebral pathology in 352 (93.4%) patients, due to trauma in 24 (6.4%) patients and due to malignancy in 1 (0.3%) patient. Among the patients, 18 (4.8%) had cervical, 10 (2.7%) thoracic, 13 (3.4%) thoracolumbar, and 336 (89.1%) had lumbar spine surgery. Intraoperative blood product transfusion was used in 72 (19.1%) patients (Table-4). The mean surgical procedure time of the patients was 181.22±48.81 minutes. The mean length of hospital stay was 4.81±2.97 (2–28) days.

In the postoperative period, PPC was identified in 30 (8%) patients, with atelectasis in 15 (4%), pneumothorax in 4 (1.1%), pneumonia in 4 (1.1%), respiratory failure in 4 (1.1%), bronchospasm in 2 (0.5%) patients, and pulmonary embolism in 1 (0.3%) patient (Table-5). PPCs were detected between postoperative first 24 hours and day 10. It was observed that the age of the patient was not statistically effective on the development of PPC. Out of the patients who developed PPCs, 3 were operated at the cervical, 1 at the thoracic, 4 at the thoracolumbar, and 22 at the lumbar level. In the postoperative period, 2 (0.5%) patients were transferred to the postoperative intensive care unit (ICU) and 29 (7.7%) patients to the Postanesthetic Care Unit (PACU) for various reasons. After the operation, a total of 4 patients, 2 patients in the ICU and 2 patients in the PACU, were intubated from the operating room. The patients who were intubated at the time of ICU admission. The patients who were intubated at the time of ICU admission were put under observation due to having a prolonged need for mechanical ventilation and hospitalization.

Table 4 Intraoperative Data

	Frequency [n (%)]
Diagnosis group	
Degenerative	352 (93.4%)
Trauma	24 (6.4%)
Neoplasm	1 (0.3%)
Diagnosis level	
Cervical	18 (4.8%)
Thoracic	10 (2.7 %)
Thoracolumbar	13 (3.4%)
Lumbar	336 (89.1%)
Intraoperative transfusion	
ES	54 (14.3%)
ES&FFP	18 (4.8%)

ES: Erythrocyte suspension, FFP: Fresh Frozen Plasma

The relationship between the presence of PPC and ASA scores was significant (p=0.000). The incidence of PPC increased with increasing ASA scores. Of the 30 patients with PPCs, 15 (50%) were scored as ASA III, 12 (40%) as ASA II, and 3 (10%) as ASA I. There was a statistically significant correlation between the presence of PPCs and ARISCAT scores (p=0.000). The ARISCAT scores were higher in patients who developed PPCs (p=0.000). The ARISCAT score was 34.13± 2.14 in the presence of PPCs, and 21.51±7.16 in patients without PPC. The mean ARISCAT score of 4 patients who were intubated during postoperative follow-up was higher than those who were extubated when the surgical procedure was completed (p=0.000). The ARISCAT score was 52.75±7.54 (42–58) in patients who were postoperatively intubated compared to 22.19±7.78 (3–62) in extubated patients. Out of the 4 patients who were postoperatively intubated, 2 were scored as ASA II, and the other 2 as ASA III (p=0.23).

In the postoperative period, one of the patients hospitalized in the intensive care unit was ASA II and the other was ASA III; these patients were in the high ARISCAT score group (ARISCAT scores: 53, 58). The ASA scores of the patients admitted to the PACU were higher than the scores of other patients. The rate of PACU admission increased with increasing ASA scores (p=0.000). Of the 29 patients admitted to the PACU, 22 were scored as ASA III, 5 as ASA II, and 2 as ASA I. The ARISCAT scores (30±12.20) of the patients in the postoperative PACU were higher than the scores of those who were postoperatively transferred to the ward (19±7.12) (p=0.000).

In the patient group with PPCs, there was a negative correlation between the ARISCAT score and the time of PPC development (p=0.169, r= -0.258). Similarly, the ASA score was negatively correlated with the time of PPC development

Table 5 Distribution of postoperative pulmonary complications according to ASA classification and ARISCAT risk index [n, (%)]

Complications	ASA I (n=73)	ASA II (n=235)	ASA III (n=69)	Low ARISCAT (n=249)	Intermediate ARISCAT (n=118)	High ARISCAT (n=10)
Atelectasis	3	5	7	4	9	2
Pneumonia	0	2	2	2	2	0
Pulmonary embolism	0	1	0	0	1	0
Respiratory Failure	0	2	2	0	1	3
Pneumothorax	0	1	3	0	3	1
Bronchospasm	0	1	1	1	1	0
Total	3(4.1%)	12 5.1%)	15(21.7%)	7 (2.8%)	17 (14.4%)	6 (60%)

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(p=0.605, r= -0.098). Patients with PPCs were found to stay longer in the hospital than those without PPCs (p=0.000). The length of hospital stay was 9.77±5.16 days in the presence of PPCs and 4.37±1.98 days in the absence of PPCs.

There was no mortality due to the development of PPCs among the study patients.

Discussion

Spine surgery is one of the most frequently performed surgeries in the world. There are several studies in the literature on the rate of pulmonary complication development and risk factors for pulmonary complications following spine surgery, but there is no study comparing the ASA classification and the ARISCAT risk index in predicting pulmonary complications. Our study found that both the ASA classification and the ARISCAT risk index were effective in predicting PPCs. The PPC development rate was observed to be high in the presence of increased ASA score and high ARISCAT risk scores.

The development of PPCs worsens the prognosis in postoperative patients [9]. In the literature, the incidence of PPCs in surgical patients shows a wide variation, considering race, geographical region, level and quality of healthcare, current diagnoses and comorbidities of patients, and the diversity of surgical procedures [2]. Studies on patients undergoing noncardiac surgery, in turn, found the incidence of PPC to be 2–19% [10,11]. It has been reported in the literature that the rates of pulmonary complications after spine surgery range from 0.9% to 5%, but the rate may differ between studies according to the method of current surgeries, surgical incision site, and the definition of pulmonary complications [12]. The rate of PPCs in patients undergoing spinal surgery were reported as 9% by Imposti et al., 15.56% by Weinberg et al., and 10.3% by Balci et al. [12-14]. In our study, an 8% incidence of PPC was found within 30 days postoperatively. This result was consistent with the incidence of pulmonary complications observed in both non-cardiac and spine surgery [11].

One of the most common causes of death following spine surgery is respiratory failure. Therefore, pulmonary complications and associated risk factors are important for preoperative planning and identification of patients at risk [15]. Various risk indexes have been proposed in the literature to estimate the risk of PPCs. The ARISCAT risk index is a scoring system easy to calculate based on seven simple variables of the patient and the surgical procedure, that provides objective results [10]. It has been reported that the ARISCAT risk index, which is also referred to as the Catalonia risk index in the literature, is effective in identifying the risk of pulmonary complications [16-18]. Our study found the ARISCAT risk index to be effective in predicting PPCs, and patients with low ARISCAT risk scores had fewer PPCs than those with medium and high-risk scores. The PPC development rate was observed to increase with increasing pulmonary risk scores.

The ASA physical status classification is an easy scoring system that has been used for a long time to estimate perioperative morbidity and mortality [19]. By providing a simple categorization of the physiological status of patients, it helps clinicians in estimating the surgical risk and it is important in predicting morbidity and mortality [20]. The ASA classification is not specific, which may cause subjective assessment and uncertain clinical interpretations among clinicians [21]. The study by Sankar et al., indicated that this scoring system had a moderate level of reliability among clinicians using the ASA scoring; although there are discussions about its reliability, it is the scoring system most frequently used by anesthesiologists to identify the perioperative risk [4,22]. Studies reporting an association between the ASA scoring system and the development of PPCs are limited, and such studies obtained different results [3,18]. Some studies in the literature emphasize the absence of a significant relationship between the ASA scoring system and the development of PPCs [5,8,23,24]. On the other hand, there are various studies indicating that the ASA classification is an important factor in the prediction of PPCs, and that there is an increase in PPCs with increasing ASA scores [16,25,26]. Our study determined that the ASA classification was important in predicting PPCs, and the PPC development rate was higher in patients with high ASA scores.

The ARISCAT risk index consists of seven independent variables: blood oxygen saturation level, recent history of upper respiratory tract infection, presence of preoperative anemia, surgical incision site, length of surgery, emergency surgery, and patient age. The blood oxygen saturation level is an objective risk indicator for cardiopulmonary function [27]. The presence of upper respiratory tract infection within the last month is a condition that may pose a risk for PPCs; the surgical procedure can be delayed in patients scheduled for elective surgeries, but care should be taken in case of pathologies requiring an emergency surgery. Preoperative anemia is a risk factor that increases perioperative mortality and morbidity rates in patients [28]. The surgical incision site is one of the two independent variables related to the surgical procedure in the ARISCAT risk index, and it is believed that the closer the incision site to the diaphragm, the greater the pulmonary risk [18]. Prolonged surgery is another factor that increases the pulmonary risk [29]. Since spine surgery was performed with a posterior approach

in our study, it was classified as a peripheral incision surgically according to ARISCAT and the incision was considered as the lowest risk. Preoperative anemia was not observed in patients undergoing elective surgery, while blood product transfusion was used in patients undergoing emergency surgery in the presence of perioperative anemia.

Age is one of the independent variables in ARISCAT risk scoring. For the ASA classification, in turn, there is no consensus on the inclusion of patient age in the scoring system. Advanced age is considered a risk factor for the development of PPC. Lung functions may deteriorate depending on age due to deterioration in lung elasticity, decrease in alveolar area and increase in dead space [16,26,30]. In contrast to studies that considered age an independent risk factor for PPCs, our study found no association between age and PPC development, similar to the result of the study by Kara et al. [18].

The Post-Anesthesia Care Unit (PACU) is a space that provides post-operative intensive care to high-risk surgical patients. The aim in PACU is to provide postoperative care, to follow the development of complications and to intervene early if they occur. The PACU is an area in which all postoperative complications, including PPCs, are common [31]. High ASA and ARISCAT scores at patient follow-ups may also be used as predictors of PPCs in PACUs. Our study determined that the ASA and ARISCAT risk scores of the patients admitted to the PACU were higher than the scores of other patients, and 75.8% of the patients admitted to the PACU were scored as ASA III. PPC development was observed in 9 patients with high ASA and ARISCAT scores among 29 PACU patients.

While ASA is a general classification system that assesses the overall health status of patients, the ARISCAT risk index is a pulmonary system-specific assessment system that produces a pulmonary risk score. In our study, both ASA classification and ARISCAT risk index were found to be effective in predicting postoperative PPCs.

The small number of patients in our retrospective and single-center study is among our limitations. Due to the absence of ASA IV and V patients in the study, the rates of pulmonary complications in patients with high ASA scores were not included in the study.

The PPC development rate is high after spine surgery. Identification of patients with risk factors for the development of PPCs is important to reduce postoperative morbidity and mortality rates. Our study, which assessed the ASA classification versus the ARISCAT risk index in the prediction of PPCs, established that both scores were effective in predicting PPCs. Since the ARISCAT index is a classification system specific to the pulmonary system, we believe that using the ASA classification together with the ARISCAT risk index would be more effective in predicting PPCs.

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Bibliometric analysis of amebiasis research

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Abstract

Aim: Amebiasis is a disease caused by protozoon *Entamoeba histolytica,* that results in amoebic dysentery. While intestinal parasites are the third leading cause of death, especially in developing countries, it has been of global concern. Bibliometric methods have been used in the parasitology discipline for more than 30 years, however there is not any bibliometric study on amebiasis in the literature. Our aim was to analyse the published literature on amebiasis by bibliometric methods.

Material and methods: A systematic evaluation of the literature using the Scopus database was made from inception to 2021. The search terms 'amebiasis', '*Entamoeba', 'Entamoeba histolytica'*, and 'amoebic dysentery' were used. The authors, publication year, title, publishing country/journal/institution, title, keywords, and citation numbers were acquired for each article. Descriptive data analysis was conducted via Microsoft Excel 2010 and Scopus database's graphics were used.

Results: Among 7,140 articles, 18.9 % of them were published open access, and 72.75 % of them were in the English language. Most of the articles were from the area of medicine. The USA, Mexico, and India were the top leading countries. The number of publications did not fall below 50 per year since 1950. There was an increasing number of citations on amebiasis research recently.

Conclusion: Amebiasis is a global concern as one of the leading infectious causes of mortality in developing countries. Bibliometric analysis has shown the growing attraction to the amebiasis research, so it will continue to be global public health issue.

Key words: amebiasis, bibliometric analysis, *Entamoeba histolytica*, bibliometrics

Introduction

Amebiasis (amoebic dysentery) is a disease caused by protozoon named *Entamoeba histolytica. E. histolytica* has morphological similarities with non-pathogenic *E. dispar,* and molecular methods should be used for identification [1]. These two parasites are thought to infect about 10% of the world's population, but 90% of these microorganisms are apathogenic *E. dispar*. Incidence of amebiasis in developing countries is quite high. Furthermore, of parasitic diseases, amebiasis is known to cause the second highest mortality in the world, after malaria [2]. In Turkey, the distribution of these two parasites was reported as 0.5–18% in different studies [2].

Amebiasis is becoming more widespread in nonendemic areas because of increased travel and emigration to developed countries. Although the majority of *Entamoeba* infections are asymptomatic, some people develop amoebic colitis and disseminated infection. Extraintestinal disseminated illness has been reported, such as liver abscess, purulent pericarditis, pneumonia, and even cerebral amoebiasis [3, 4].

According to our literature search, the bibliometric evaluations in the context of this emerging and re-emerging disease have never been discussed before. The findings of our study could be beneficial in determining amebiasis research priorities and determining the importance of scientific research on this infection.

Material and methods

Data sources

The Elsevier's Scopus bibliometric database was used in this study. A systematic evaluation of the literature resulted in extensive use of the Scopus database from inception to 2021. We used the search terms 'amebiasis', '*Entamoeba*', '*Entamoeba histolytica*', and 'amoebic dysentery'. Only research articles have been used for further analysis. All digital searches were done on February 13, 2021. The publications published in the year 2022 were excluded from the search because the year 2022 is not completed, and all data for that year were not available.

Data collection

A total of 8,135 publications records were obtained from the Scopus database. The following records were acquired for every article: authors, publication year, publishing institution/ country/journal/, title, keywords, and citation numbers.

Analyses and visualizations

Using Microsoft Excel 2010, the data in the tables were converted to absolute values (percentage and frequency). There were no relative frequencies utilized. There were no sophisticated statistical procedures applied, such as mean, median, and fashion, dispersion measures, standard deviation, or statistical tests. The visualizations from the Scopus database were also utilized.

Free versions of the Dimension programme (https://app.dimensions.ai/) and the VOSviewer were used for analysing and visualising the co-authorship between countries and co-citations.

Ethical aproval

The study complied with the Helsinki Declaration, which was revised in 2013. Ethics committee approval is not required, as there is no human or animal research.

Results

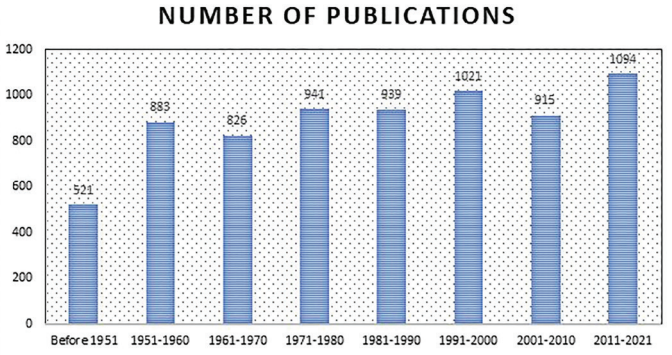
Our Scopus database search for publications on amebiasis research globally to 2021 yielded 7,140 articles. 1,351 (18.9%) of them were published open access, and 5,195 (72.75%) of them were in the English language.

The first publication was published in the United States of America (USA) in 1892 [5]. Fifty-one per cent (n=5264) of the articles were from the area of medicine. There were a further nine subject areas on amebiasis research. Immunology and microbiology (n=2464), biochemistry, genetics and molecular biology (n=1215), agricultural and biological sciences (n=483), and veterinary science (n=240) were other main subject areas.

The number of publications did not fall below 50 documents per/year from 1950. The year 2000 was the year with the most publications (213 publications) (Figure 1). 5,193 (72.73%) of the articles were in the English language. The other preferred languages were Spanish (n=573, 8.02%), French (n=431, 6.03%), German (n=132, 1.84%), and Portuguese (n=123, 1.72%).

The USA was found to be the most scientific country with 1,222 (17.11%) articles on amebiasis. Mexico (n=885, 12.39%), India (n=543, 7.6%), Japan (n=368, 5.15%), Germany (n=279, 3.9%), the United Kingdom (n=276, 386%), France (n=193, 2.7%), Canada (n=144, 2.01 %), Israel (n=143, 2%), and Brazil (n=118, 1.65%) were the top leading countries on amebiasis research. Turkey ranked 15th with 60 publications. The publications originated from over 100 countries.

Figure 1 - Number of publications by the years



Centro de Investigacion y de Estudios Avanzados from Mexico has been seen to be the leading institution in this field with 393 (5.5%) publications, and most of the leading institutions on amebiasis research were from Mexico. The other leading institutions are also summarized in Table 1. William A Petri (from the University of Virginia School of Medicine, United States) (n=147), Tomoyoshi Nozaki (from the Graduate School of Medicine, Japan) (n=126), and Alok Bhattacharya (from Jawaharlal Nehru University, India) (n=108) were the top researchers in the field.

Table 1 The leading instutions on amebiasis research.

Instutions/ Country	n (total=7140)	Frequency
Centro de Investigacion y de Estudios Avanzados/Mexico	393	5.5
Instituto Politécnico Nacional/Mexico	235	3.29
Instituto Mexicano del Seguro Social/ Mexico	185	2.59
Jawaharlal Nehru University	160	2.24
Bernhard Nocht Institut fur Tropenmedizin Hamburg/ Germany	130	1.82
University of Virginia/ USA	124	1.73
Universidad Nacional Autónoma de México, Facultad de Medicina/Mexico	120	1.68
London School of Hygiene & Tropical Medicine/Unıted Kingdom	118	1.65
National Institute of Infectious Diseases/ USA	108	1.51
Universidad Nacional Autónoma de México/Mexico	103	1.44

Archivos de Investigacion Medica (n=288), American Journal of Tropical Medicine and Hygiene (n=248), Molecular and Biochemical Parasitology (n=229), Experimental Parasitology (n=208), and Archives of Medical Research (n=140) were the top five journals on amebiasis research (Figure 2).

Figure 2 - Comparison of number of the documents

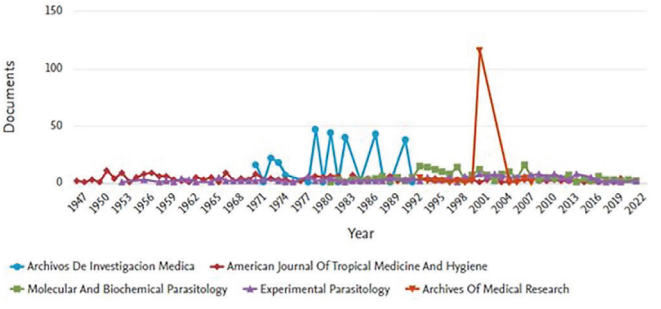


Table 2 The examination of the top 10 documents in terms of citations (6-15).

Document title /Reference number	Authors; Year	Source	Number of citations
A new medium for the axenic cultivation of entamoeba histolytica and other entamoeba [6]	Diamond et al.,1978	Transactions of the Royal Society of Tropical Medicine and Hygiene	1537
The genome of the protist parasite Entamoeba histolytica [7]	Loftus et al.,2005	Nature	676
Problems in recognition and diagnosis of amebiasis: Estimation of the global magnitude of morbidity and mortality [8]	Walsh, J.A.,1986	Reviews of Infectious Diseases	513
A clonal theory of parasitic protozoa: The population structures of Entamoeba, Giardia, Leishmania, Naegleria, Plasmodium, Trichomonas, and Trypanosoma and their medical and taxonomical consequences [9]	Tibayrenc et al,1990	Proceedings of the National Academy of Sciences of the United States of America	498
A Redescription of Entamoeba Histolytica Schaudinn, 1903 (Emended Walker, 1911) Separating It From Entamoeba Dispar Brumpt, 1925 [10]	Diamond et al.,1993	Journal of Eukaryotic Microbiology	449
The analysis of 100 genes supports the grouping of three highly divergent amoebae: Dictyostelium, Entamoeba, and Mastigamoeba [11]	Bapteste et al.,2002	Proceedings of the National Academy of Sciences of the United States of America	310
Simultaneous Detection of Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum in Fecal Samples by Using Multiplex Real-Time PCR [12]	Verweij et al.,2004	Journal of Clinical Microbiology	303
The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite Entamoeba histolytica [13]	Tovar et al.,1999	Molecular Microbiology	282
Role of adherence in cytopathogenic mechanisms of Entamoeba histolytica. Study with mammalian tissue culture cells and human erythrocytes [14]	Ravdin &Guerrant, 1981	Journal of Clinical Investigation	265
Techniques of axenic cultivation of Entamoeba histolytica Schaudinn, 1903 and E. histolytica-like amebae [15]	Diamond, L.S.,1968	The Journal of parasitology	262

National Institute of Allergy and Infectious Diseases (n=451), National Institutes of Health (n=316), Consejo Nacional de Ciencia y Tecnología (n=243), U.S. Department of Health and Human Services (n=159), Japan Society for the Promotion of Sciences (n=451) were the top funding sponsors. Table 2 summarized the top 10 documents in terms of citations [6-15].

Figure 3 - Number of the citations by the years

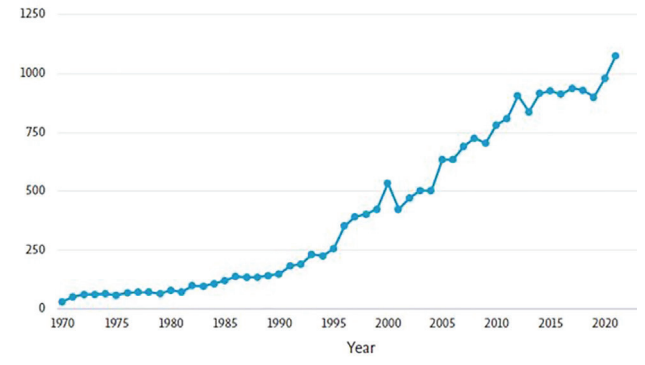


Figure 4 - Co-authorship analysis

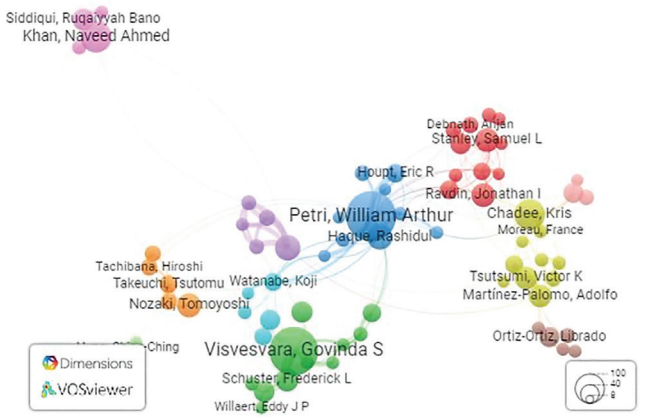
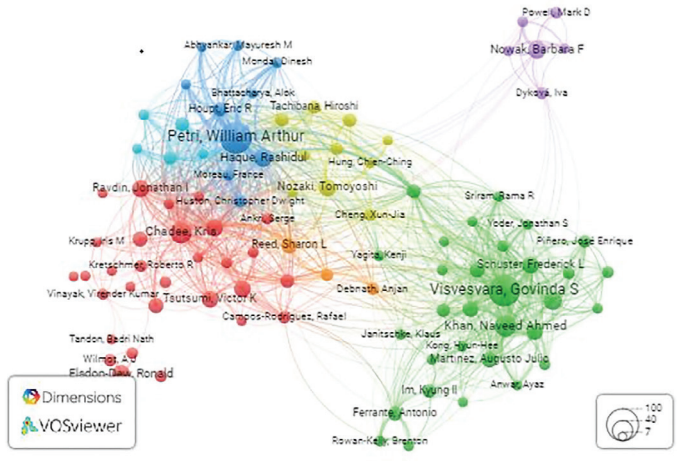


Figure 5 - Citation Analysis



There was an increasing number of citations on amebiasis research recently (Figure 3). Furthermore, Figure 4 demonstrates the co-authorship analysis and Figure 5 shows co-citation analysis.

Discussion

Our study analysed the bibliometric data on amebiasis, primarily in the literature. According to our analysis, the research on amebiasis is active, interdisciplinary, and collaborative in nature.

Entamoeba histolytica is a unicellular extracellular protozoan parasite that infects the human intestinal tract and results in bloody diarrhea and colitis. Amebiasis can also cause extraintestinal abscess formation in the liver, lung, and brain. Intestinal parasites are the third leading cause of death, especially in developing countries. Developed countries have also been affected since high-risk groups including tourists and

immigrants are candidates for infection. Therefore, amebiasis has been of global concern [16, 17].

Bibliometric analysis has analysed the published literature using quantitative and qualitative metrics, so that academic productivity might be revealed objectively [18]. Bibliometric methods have been used in the parasitology discipline for more than 30 years, but it is still at an early stage considering the available literature. Its primary aim is to reveal trending research topics via searching major literature databases [19].

Analysis of the literature research on amebiasis shows that most of the articles were written in English. English is accepted as the de facto universal language of science, and so most of the cited articles are from English written journals [20].

Another highlighted point of our bibliometric analysis reveals that entamoeba research is primarily the subject of the medical field; however, nine other health disciplines including the veterinary field have analysed this parasite infection according to the literature. Research on parasite infections has been the subject of many health disciplines due to the characteristics of the disease. Humans are natural hosts of the entamoeba histolytica, and there is not a unique animal model that mimics the cycle of the disease. However, animal models and experimental ex vivo systems are the only solution to conduct research on amebiasis. Furthermore, it is a disease in which innate and adaptive immune responses play a key role in the course of the disease, from asymptomatic disease to its fatal form. Technological advancements have resulted in progress for diagnostic tools and a better understanding of the pathogenesis of the infection [16, 21]. For instance, recently it has shown the interaction of human microbiota and entamoeba histolytica, which plays a major role in the disease course [22].

These developments are closely related to interdisciplinary collaborative studies, which also reflect the results of our bibliometric analysis.

Amebiasis has been highly examined since 1950, and over 50 publications per year have been published. Since the modern pathogenesis of the infectious disease was developed in the early 50s, dating to the post-World War II era, entamoeba research might also be popular [21]. In particular, the years from 2011 to 2021 represent the most prolific era in that field regarding publications.

Considering contributions of literature, the USA is the leading country regarding publications. In general, the USA is one of the most productive countries in parasitology [23]. According to our results, the second and third prolific countries in this field are Mexico and India. The most prolific centre in the field is also from Mexico, which consists of 5.5% of all publications. Parallel to our results, Mexico and India are the most prolific countries regarding studies on amoebic liver abscess [24]. These results are compatible with the epidemiological data of amebiasis, which is commonly seen in developing countries and tropical areas [17]. Researchers of the most affected countries have also contributed significantly to the literature.

Another important finding of our research revealed that more than 100 countries have contributed to the literature on amebiasis, which has shown the global importance of this parasite infection. Turkey is amongst those countries, contributing to the field with a growing number of publications recently [25].

Considering journals that were published mostly on amebiasis research, most of them are parasitology journals. Furthermore, most of the funding centres are national institutes of the countries. This result has shown that local authorities in the countries have attached importance to this global health issue. Since the early 1970s, the number of citations has increased regularly. In the most cited studies, there is not a regular pattern of data and year distribution regarding citations. The most cited study is from 1976, with the next most highly cited studies mainly occurring after the 1990s. While the oldest most cited studies have analysed the pathogenesis and diagnosis of amebiasis, the newest studies have examined the genomic profile of this parasite. Technological advancement in molecular biology might steer the more recent studies [26].

Additionally, the most cited articles were written by a group of researchers. This has shown the importance of collaborative work in the field.

Although amebiasis research is still developing and being contributed to by many fields, there are still relatively few studies of bibliometric analysis of parasitology. Hence, there is no documented study on amebiasis or entamoeba histolytica using bibliometric analysis. Only one bibliometric analysis searching for liver abscess has emphasized this infective agent amongst risk factors [24].

Conclusion

Amebiasis has been the subject of a wide range of health disciplines. Since the cumulative data on molecular biology has increased in the last 50 years, the diagnostic and therapeutic developments on amebiasis research have changed the content of the studies so recently, the most cited articles are related to these developments. Amebiasis is still a global concern as one of the leading infectious causes of mortality in developing countries, so it will continue to be a prominent topic of global public health.

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Treatment of the fistula tract with laser ablation in high anal fistulas

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Abstract

Aim and introduction: Considering the recurrence and fecal incontinence rates in high anal fistulas, surgical treatment of anal fistulas is a challenging process, although many treatments have been defined today. The aim of our study is to evaluate the long-term results of laser ablation of the fistula tract in high anal fistulas.

Material and methods: The files of patients who underwent laser ablation of the fistula tract due to high anal fistula between June 2020 and January 2022 were evaluated retrospectively. Moreover, their postoperative complications, preoperative and postoperative Cleveland fecal incontinence scores (CCFFSI score), postoperative first day and first-week visual analog scale (VAS) scores, follow-up times, and recurrence rates were analyzed.

Results: 26 patients were included in the study. The mean follow-up period was 39.88±14.34 weeks, and the postoperative first and 7th day VAS scores were 4.61±1.41 and 0.8±1.02, respectively. Preoperative and postoperative CCFI scores were calculated as 1.8±1.41, 1.65±1.32, respectively. Recurrence was observed in 7 patients postoperatively. Postoperative anal abscess developed in 1 patient.

Conclusion: Although laser ablation of the fistula tract can be safely performed as a technique that does not affect incontinence, recurrence rates should also be considered. Furthermore, more extensive randomized prospective studies on this technique should be performed.

Key words: high anal fistula, laser ablation of fistula tract, laser

Introduction

Anal fistulas occur as a result of chronic infection of cryptoglandular structures in the anal canal and almost always require surgical treatment [1]. Though many surgical techniques are defined for the treatment of anal fistula today, when long-term data are evaluated, there is no single gold standard treatment method [2]. In terms of sphincter-preserving techniques, rectal mucosal advancement flaps, LIFT (ligation of the intersphincteric fistula tract), VAAFT (video-assisted anal fistula treatment) and laser ablation procedures (LAFT) are some of these methods [3-6].

According to Milligan and Morgan, anal fistulas are divided into high and low according to the closure rate of the puborectal muscle and external sphincter [7].

In our study, we aimed to evaluate the results of laser ablation treatment applied as a new technique in patients with high anal fistula.

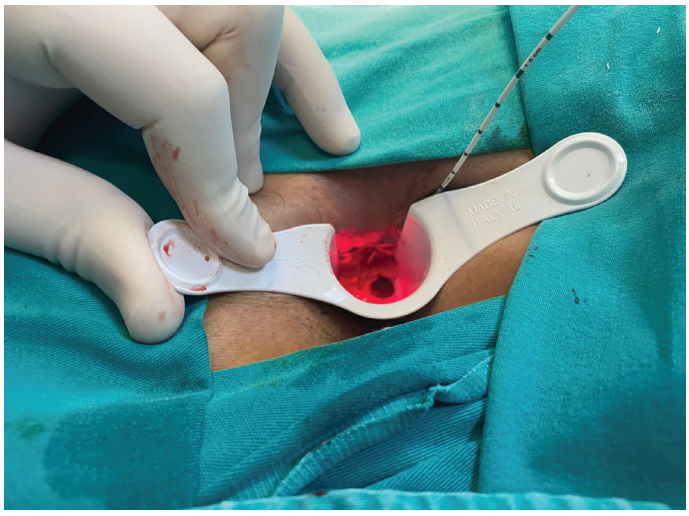
Material and methods

The files of patients who underwent laser ablation of the fistula tract due to high anal fistula between June 2020 and January 2022 in Aydın Surgery Clinic at Turkey, Aydın were evaluated retrospectively. Moreover, their postoperative complications, preoperative and postoperative Cleveland fecal incontinence scores (CCFFSI score), postoperative first day and first-week visual analog scale (VAS) scores, follow-up times, and recurrence rates were analyzed. Patients with a follow-up period of less than 6 months were excluded from the study in order to more clearly evaluate the postoperative recurrences of the patients. Patients with Crohn's disease, cancer patients, patients who developed fistula secondary to trauma, and patients who were treated for anal fistula due to recurrence, and low anal fistula were excluded from the study.

Surgical method

Enema was applied to the patients 2 hours before the operation. Preoperatively, 1 gr ampicillin/sulbactam was administered intravenously. After spinal anesthesia, the patients were prepared in lithotomy or jack knife position according to the location of the external of the fistula. By revealing the external fistula tract, hydrogen peroxide was injected through the external sphincter orifice and the internal sphincter opening was revealed with the help of anal retractor. The fistula tract was determined with a stylet. Following that, the brush stylet fistula tract was debrided. Then, the 1000-µm-diameter radial laser probe (FiLaC®) was advanced and removed from the internal of the fistula (Figure 1).

Figure 1 - Treatment of the fistula tract with laser ablation in high anal fistulas



According to the size of the 1470 nm 10 watt energy fistula tract, an average of 12-18 shots were made along the fistula tract in total. The external orifice was excised and left open for drainage.

Statistical analysis

For data evaluation, the SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical packaged program was used. The variables were stated using the mean±standard deviation, percentage and frequency values. Kolmogrov Smirnov test was performed to evaluate the homogeneity of the data. In the analysis of data, Mann–Whitney U test were used for the comparison of CCFFSI and VAS scores. $P < 0.05$ was considered statistically significant.

Results

A total of 26 patients were included in the study, of which 17 (65.4%) were male and 9 (34.6%) were female. The mean age of the patients was calculated as 40.2±11.2. High fistulas were included in the study and 18 (69.2%) were transsphincteric and 8 (30.8%) were extrasphincteric. The follow-up period of the patients was calculated as 39.88±14.34 weeks. Recurrence was detected in 7 (26.9%) of the patients included in the study. When the VAS scores were examined, it was 4.61±1.41 on the postoperative 1st day and 0.8±1.02 on the postoperative 7th day (p<0.001) (Table 1). When considered as a complication, perianal abscess developed in one patient on the postoperative 4th day (3,8%). Globe vesicale developed in one patient one postoperative first day (3,8%) (Table 2).

Table 1 Score and general definitions

Age	40.2±11.2	
CCFFSI score preoperative	1.8±1.41	
CCFFSI score postoperative 1.week	1.65±1.32	
Vas score postoperative 1.day	4.61±1.41	
Vas score post op 7.day	0.8±1.02	
Follow-up time	39.88±14.34(24-75)	
Recurrence	7(26.9%)	
Gender	17(65.4%)	
man	9(34.6%) woman	
Fistula type	18(69.2%) transsphincteric	8(30.8%) extrasphincteric

Table 2 Complications

Periperianal abscess	1 (3,8%)
Globe vesicale	1 (3,8%)

Table 3 Preoperative and postoperative scores

Preoperative CCFI score 1.8±1.41	Postoperative CCFI score 1.65±1.32	p=0.1
Postoperative 1. day VAS 4.61±1.41	Postoperative 7. day VAS 0.8±1.02	p<0.001

Considering the CCFFSI scoring, while the preoperative value was 1.8±1.41, it was calculated as 1.65±1.32 at postoperative 1st week (p=0.1) (Table 3).

Discussion

Although many sphincter sparing techniques have been described for high anal fistulas, none of these techniques have taken their place in the literature as the gold standard treatment. The fibrin glue technique, which is one of these techniques, recurrence rates were found to be high [8,9]. In a meta-analysis study conducted on another technique, the LIFT technique, the success rates were reported to be around 76%, and its protective effect on incontinence was stated to be present [10]. In a another study about modified LIFT technique Celayir et al [11] reported success rate about 87.5%. In a study on rectal mucosal advancement flaps, the recurrence rate was reported as 23% [12]. In the literature, factors affecting recurrence in anal fistula have been reported as recurrent anal fistulas, diabetes, smoking, and immunosuppressive diseases [13]. Since our study was retrospective, the risk factors of the patients could not be evaluated. Although there are studies showing recurrence rates in the range of 18-75% after the LAFT technique, recurrence was found in 7 patients in our study, and our recurrence rate was found to be 26.9% [14,15].

Anal fistula poses a serious problem after surgery, particularly after surgery for high fistulas. Ege et al. found no significant difference between preoperative and postoperative incontinence scores after hybrid seton surgery in high anal fistulas [16]. There are a few studies in the literature that evaluate with the incontinence scoring system after the LAFT technique. In a study on the LAFT technique, Giamundo et al [17] found no significant difference between preoperative and postoperative CCFFSI scores. Similarly in our study, no significant difference was found between the preoperative and postoperative 1st week CCFFSI scores. In another study Wilhelm et al [18] reported that

no continence developed in their patients after LAFT. Hence, it can be said that the LAFT technique is a reliable technique for incontinence.

Patient comfort and quality of life are important after surgery in anorectal diseases. There are many studies available to detect this aspect. In particular, pain can be encountered as a distressing symptom postoperatively after fistula surgeries. In a study conducted by Giamundo et al [17] on the LAFT technique, the mean VAS values were found to be 4 in the VAS surveys to evaluate the preoperative and postoperative pain status, and no change was detected [17]. In our study, on the other hand, while VAS values were found to be 4.61±1.41on postoperative 1st day, on postoperative 7th day it was found to be 0.8±1.02 and a significant decrease in pain level was detected. In terms of postoperative pain, LAFT can be considered as a comfortable method. For more detailed post-operative comfort, quality of life questionnaires such as SF-36 are required. However, as our study was a retrospective study, a questionnaire could not be applied to the patients.

Conclusion

Although the LAFT technique is not the gold standard treatment for high anal fistulas, it can be considered as a method that can be preferred in terms of preserving sphincter functions and postoperative pain comfort; however, surgical recurrence rates should also be considered. We believe that prospective studies with other techniques are needed to access more detailed information.

Study limitations: The main limitations of this study are that it is retrospective, and low number of patients.

Ethics Committee Approval: The study protocol was approved by Institutional Research Ethic committee.

Informed Consent: All patients were informed about the procedure, and certificate of consent was taken for every patient.

Disclosures: There is no conflict of interest for all authors.

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Association between serum 25-hydroxyvitamin D concentration and severity of seasonal allergic rhinitis in Karaganda region (Kazakhstan)

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Abstract

Background: Vitamin D deficiency (VDD) is the one of the major public health problem affecting approximately one billion people all over the world. In recent years, the relationship of allergic diseases with a low concentration of vitamin D has been studied worldwide. An association has been found between small count of serum vitamin D and the development of immune disorders. Patients with allergic disorders and, in particular, with respiratory allergy are susceptible for VDD.

Objective: The study was aimed to assess the levels of serum 25-hydroxyvitamin D (25(OH)D) and their associations with the severity of seasonal allergic rhinitis in the Karaganda region (Kazakhstan).

Material and methods: This cross-sectional study included 416 patients with seasonal allergic rhinitis aged 18-65 years (mean age 39±8 years), 267 of whom were females. VDD was defined as serum concentrations of 25(OH)D below 20 ng/ml.

Results: The median concentration of 25(OH)D in blood serum was below the reference threshold (20 ng/ml) and amounted to 16.1 ng/ml. 75% of patients with seasonal allergic rhinitis had VDD and this was common in all age categories. VDD was more prevalent in female patients (82.8%) as compared with the male patients (61.1%) (p<0,01). Of interest is the fact that low serum 25(OH)D concentration correlated with the severity of symptoms (r =-0.94 and r =-0.67).

Conclusion: According to our study, the significant part of patients with allergic rhinitis residing in Karaganda region (Kazakhstan) had deficient status of 25(OH)D and this correlated with the severity of symptoms.

Key words: adults, vitamin D deficiency, Karaganda region, Kazakhstan

Introduction

Vitamin D deficiency (VDD) is the one of the major public health problem affecting approximately one billion people all over the world. In recent years, the relationship of allergic diseases with a low concentration of vitamin D has been studied worldwide. An association has been found between small count of serum vitamin D and the development of immune disorders [1]. Patients with allergic disorders and, in particular, with respiratory allergy are susceptible for VDD [2].

It is known, that the participation of vitamin D is related to development of allergic processes. Vitamin D is considered as an immunomodulatory [3, 4] acting on dendritic cells (DC), macrophages, T-cells and B-cells [1, 5, 6]. Activated B-lymphocytes, T-lymphocytes and myeloid APCs can synthesize biologically active calcitriol from 25-hydroxyvitamine D3 (inactive precursor) [7]. Vitamin D suppresses dendritic cell differentiation, maturation, and immunostimulation by inhibiting the expression of class II MHC molecules [8]. Thus, physiological quantities of vitamin D maintain the level of tolerogenic dendritic cells producing Interleukin-10 (IL-10) [1].

Vitamin D contributes to maintaining a balance between T-helpers type 1 (Th1) and type 2 (Th2) [4]. Several researches have shown that vitamin D deficiency can be the reason to increased Th2 and decreased Treg and IL-10 [9, 10]. Adequate quantity of vitamin D in the blood contribute to the suppression of IgE formation, as well as enhance the secretion of IL-10 by B-lymphocytes [6, 7].

Karaganda region (Kazakhstan) is characterized by long winter, with decreased insolation and high levels of pollutants in the atmospheric air, exceeding the maximum permissible concentrations that might further deteriorate VDD [11].

The aim of this study was to assess the serum 25-hydroxyvitamin D levels (25(OH)D) and their associations with the severity of seasonal allergic rhinitis in Karaganda region (Kazakhstan).

Material and methods

Study design

We conducted a cross-sectional trial. It was carried out from July 1, 2019 to September 31, 2019 at Divera Allergy Center in Karaganda (Kazakhstan).

Study object

The study included 416 patients aged 18-65 years (mean age 39±8 years, 267 of whom were females) with a diagnosis of seasonal allergic rhinitis. Population distribution by age groups: 18-39 years – 223, 40-59 years – 136, >60 years – 57. Verification of the seasonal allergic rhinitis diagnosis was performed on the basis of the anamneses and complaints of patients, an objective examination, and the collection of an allergy anamnesis. Confirmation of diagnosis was performed by skin test. Two weeks prior to the study, patients were excluded from taking all medications for allergic rhinitis.

The exclusion criteria were: presence of any acute or chronic severe somatic pathology, including hepatic or renal disease, metabolic bone disease, type 1 diabetes, malignancy, history of recent immobility for a period of more than one week, pregnancy and lactation, current dieting or consumption of multivitamin supplements containing vitamin D or its combinations.

Questionnaire

A questionnaire was employed to assess the severity of symptoms of seasonal allergic rhinitis. Self-assessment was based on an adapted questionnaire described by Pfaar et al. [12]. The questionnaire included evaluating nose itch, nasal congestion, watery/mucous nasal discharge, sneezing, itchy eyes, and watery eyes. An assessment of each symptom was expressed on a 3-point scale depending on the severity of the manifestation: 0 – no symptoms; 1 – mild degree (the symptom is minimally pronounced, occurs 1-3 times a week; the state of health is of no concern); 2 – moderate degree (the symptom is pronounced, has a frequency of manifestation 4-5 times a week, moderately affects well-being and sleep); 3 – severe degree (the symptom is very pronounced, has a systematic and persistent character on a daily basis, imposes a pronounced impact on sleep and labor activity). The maximum score constitutes 18 points, which is interpreted as a severe degree of manifestations. A severity score of 1 to 6 suggests mild severity, 7-12 corresponds to a moderate severity, and ≥13 points suggest severe degree of manifestation (Author's Certificate for Invention No. 14535 of January 19, 2021).

Laboratory analyses

Blood sampling for the vitamin D content was conducted from 8:00 am to 12:00 pm (noon) at a network of government-licensed laboratories (Olymp). The content of vitamin D in blood serum was determined via the chemiluminescence method on Beckman Coulter DxI automatic modular analyzer (USA). The normal range for serum vitamin D levels was at least 30 ng/mL [4,11]. The results in the range of 21-29 ng/mL were regarded as vitamin D insufficiency, and below 20 ng/mL as vitamin D deficiency [12].

Statistical analysis

Statistical analyses were carried out in Statistica 13 for Windows. At the first stage, the type of data distribution was determined and descriptive statistics of numerical variables were carried out. Since the data distribution was different from normal, descriptive statistics were generated by calculating the median and corresponding boundaries of the 25th and 75th percentiles. Mann-Whitney test was applied for qualitative variables to find differences in comparison groups. Spearman coefficient of correlation was applied to determine the correlation between the groups. The critical level was established on the probability of error of the first type not more than 5% ($\alpha < 0.05$)

Ethics

All the study participants signed voluntary informed consent before participation. The study protocol was approved by the Ethics Committee of the Non-Profit Joint-Stock Company Karaganda Medical University (Protocol No 14, March 11th, 2019).

Results

The median quantities of 25(OH)D in blood serum in Karaganda was less than the reference limit (20 ng/ml) and amounted to 16.1 ng/ml (Figure 1).

Figure 1 - Vitamin D concentration in blood in patients with seasonal allergic rhinitis

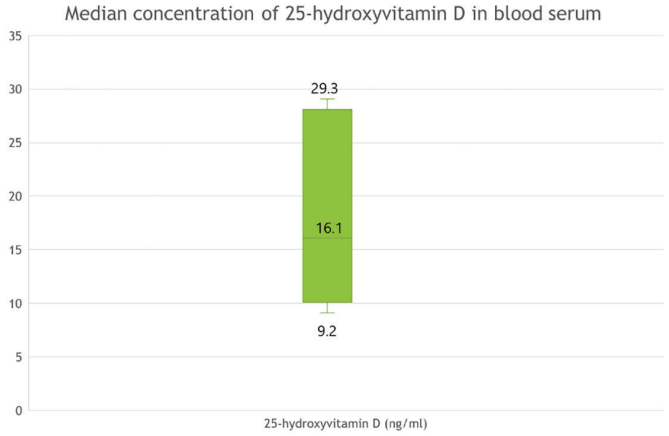


Figure 2 - Prevalence of vitamin D deficiency among patients with seasonal allergic rhinitis

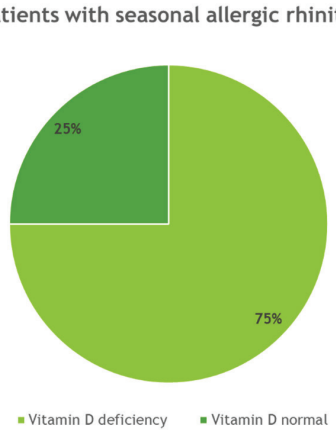


Figure 3 - Prevalence of vitamin D deficiency among different age categories

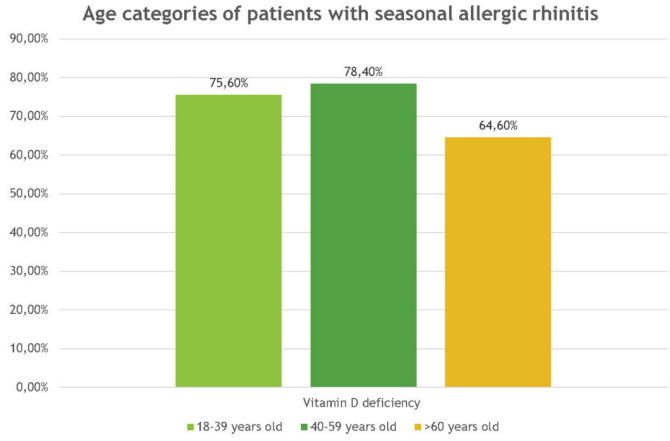
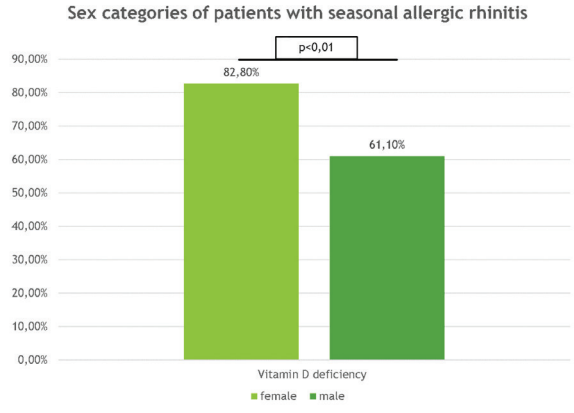


Figure 4 - Prevalence of vitamin D deficiency among men and women with seasonal allergic rhinitis



Most people in our study (75% of patients with seasonal allergic rhinitis) had VDD and this was common in all age categories: 75.6 % of 18-39 years old, 78.4 % of 40-59 years old, and 64.6 % of >60 years old (Figure 2, 3). There were no particular differences in concentrations of 25(OH)D in the age groups in blood serum at the cut-offs of <20 ng/mL.

In addition, VDD was more prevalent in female patients with seasonal allergic rhinitis (82.8 %) as compared with the male patients (61.1 %) (p<0,01) (Figure 4). Females had deficient vitamin D status a quarter more often than males.

Of interest is the fact that low serum 25(OH)D concentration correlated with the severity of symptoms: nasal congestion and nasal discharge (r =-0.94), sneezing, itchy nose and eyes, tearing (r =-0.67) (Figure 5 a, b, c, d, e, f).

Figure 5a - correlation the nasal congestion with vitamin D concentration

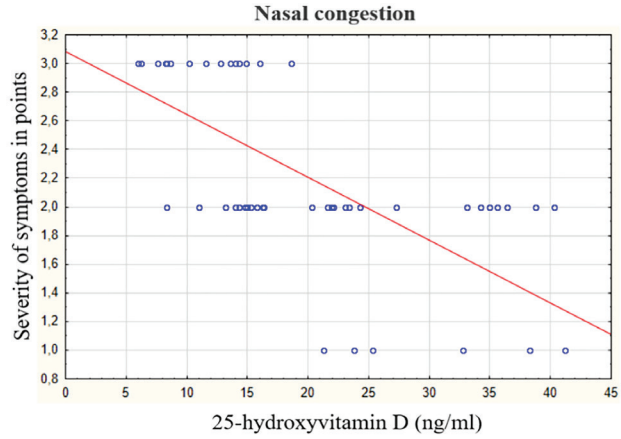


Figure 5b - correlation the nasal discharge with vitamin D concentration

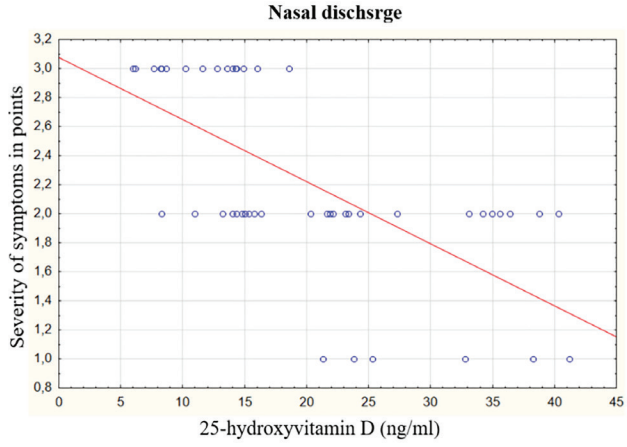


Figure 5c - correlation the sneezing with vitamin D concentration

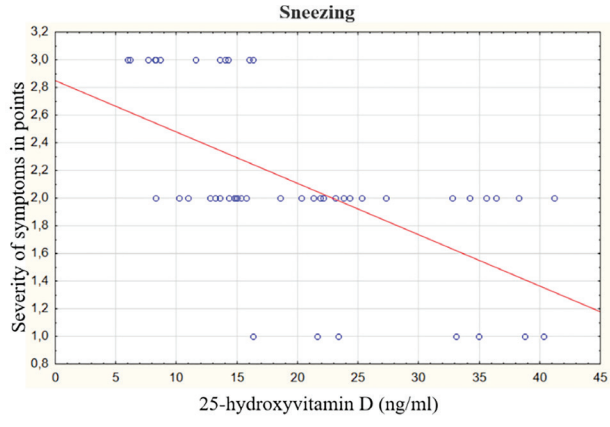


Figure 5d - correlation the itchy nose with vitamin D concentration

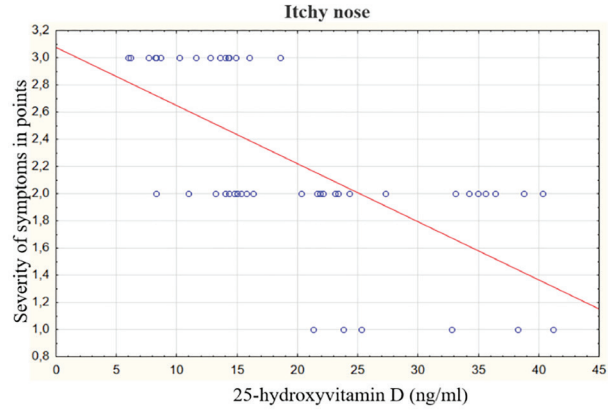


Figure 5e - correlation the itchy eyes with vitamin D concentration

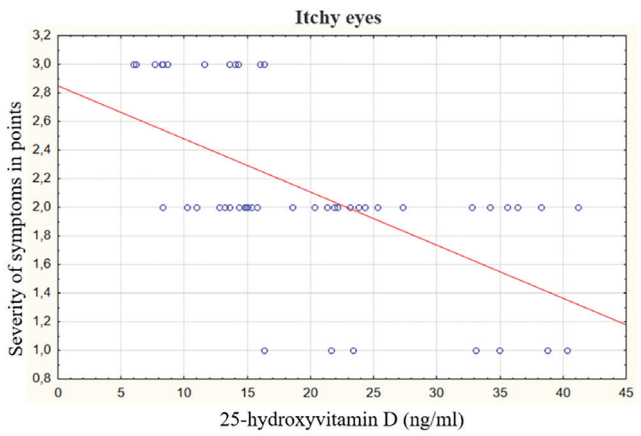
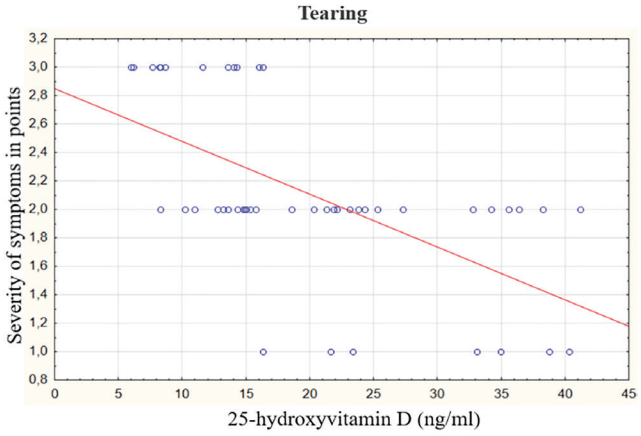


Figure 5f - correlation the tearing with vitamin D concentration



Discussion

This study aimed at evaluation the serum 25-hydroxyvitamin D levels (25(OH)D) and their associations with the severity of seasonal allergic rhinitis in Karaganda region (Kazakhstan). The major finding of this study was a high rate of VDD as three quarters of study participants had serum 25(OH)D concentrations below 20 ng/mL. It`s only 25 % of them had optimal levels of serum 25(OH)D defined as 30 ng/mL (75 nmol/L). Female gender was the major contributing factors for VDD.

In this study a connection was found to the vitamin D levels in the body and severity of the allergic rhinitis. The lower the vitamin D level in the serum, more severe were the symptoms. A study conducted by Arshi et al. (2012) showed that it was important to assess vitamin D levels in patients suffering from allergic rhinitis, whereas women were less likely to develop vitamin D deficiencies [13]. Bukhari et al. (2020) found that a lack of vitamin D is closely linked to a non-controlled allergic rhinitis [14].

In recent years, there has been a growing amount of researches on the interplay of vitamin D levels in the blood and the allergic diseases disease progression and severity [15–18]. The existence of a correlation between serum vitamin D concentration and allergic rhinitis status has been proven by various studies. Studies prove that allergic rhinitis is more common in patients with severe vitamin D deficiency in the blood [1, 10, 13, 19]. These studies suggest that vitamin D deficiency may cause eosinophil activation and release of high levels of eosinophilic cationic protein [20], which in turn has an effect on nasal mucosal inflammation in patients with allergic rhinitis [21].

Various clinical studies have shown that vitamin D supplementation is important in the prevention of allergic rhinitis, asthma and other allergic diseases [10]. The study of vitamin D as an additional treatment for allergic rhinitis in children with sensitization to grass pollen during the dusting season identified a significant reduce in the symptoms of the disease and a decrease in the use of drugs. These results are straight confirmation of the efficacy and safety of vitamin D 1000 IU as an additional therapy for grass pollen allergy in allergic rhinitis patient during pollen season [22, 23].

This study has some limitations, as we have only limited data in the Karaganda region. The results of the work can serve as a basis for further studies in the area of the study of the vitamin D levels of patients with allergic rhinitis in Kazakhstan and elsewhere. In addition, we have launched a project dedicated to the study of the effectiveness of allergen-specific immunotherapy in combination with vitamin D in patients with allergic rhinitis. Nevertheless, the results of this study can serve as recommendations for representatives of practical healthcare to diagnose all patients with allergic rhinitis for vitamin D status.

Conclusion

Nowadays, VDD remains an unresolved problem in Kazakhstani patients with seasonal allergic rhinitis. In fact, vitamin D is a prohormone with immunomodulating properties. According to our study, the significant part of patients with allergic rhinitis residing in Karaganda region of Kazakhstan had deficient status of 25(OH)D and this correlated with the severity of symptoms. The outcomes of our study could be of interest for both clinical physicians and public health professionals, who could envisage preventive strategies to tackle this problem.

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The role of ANDC early warning score in predicting prolonged hospitalization in SARS-Cov-2 infected patients

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Abstract

Aim: To evaluate the ability of the age, neutrophil-to-lymphocyte ratio, D-dimer, C-reactive protein (ANDC) score to predict prolonged hospitalization in SARS-CoV-2-infected patients.

Material and methods: This is a prospective and observational study conducted with patients hospitalized due to SARS-CoV-2 infection. The patients were divided into expected and prolonged hospitalization groups according to their length of hospital stay, and those who were hospitalized for seven days or longer were included in the prolonged hospitalization group. The receiver operating characteristic analysis was performed and the DeLong equality test was applied to compare the area under the curve values of the investigated parameters. Their odds ratios were also calculated.

Results: The study included a total of 397 patients. The median length of hospital stay was 8 days (25th-75th percentiles: 5-13). The univariant analysis revealed significant differences in the ANDC scores between the expected and prolonged hospitalization groups (101 (80.1-127) versus 114 (94.3-141), p<0.001, Mann-Whitney U test). The area under the curve value of the ANDC score in the prediction of prolonged hospitalization was 0.609 (75.91% sensitivity, 42.94% specificity, 62.3% positive predictive value, and 58.9% negative predictive value at a cut-off value of 93.5), and the odds ratio was 2.6.

Conclusion: Our results suggest that ANDC score is a predictor of prolonged hospitalization in SARS-CoV-2-infected patients. However, multicenter studies are needed to confirm our findings in larger samples.

Key words: ANDC score, COVID-19, SARS-CoV-2, length of stay, prolonged hospitalization

Introduction

In December 2019, pneumonia cases of unknown cause were detected in Wuhan, China. The sequence analyses performed on the lower respiratory tract samples taken from these cases revealed a new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease caused by this virus was named coronavirus disease 2019 (COVID-19). With the rapid spread of the disease, a pandemic was declared on March 11, 2020. The pandemic has placed an extra burden on the healthcare system worldwide [1], with the patient load exceeding the current bed and staff capacity, especially during peak periods. Early warning systems have been discussed in the literature in order to determine high-priority cases and use the available beds and health capacity effectively [2].

Researchers have investigated many early warning scores (EWSs) as a predictor of poor outcome. Scoring systems, such as CURB-65, CURB, and pneumonia severity index have been shown to predict the severity of pneumonia [3,4]. Laboratory parameters have been used to predict severe disease and need for supportive care. Hematological parameters and their combinations were primarily investigated for this purpose, as they are

easily accessible and inexpensive. Higher D-dimer, C-reactive protein (CRP), and neutrophil-to-lymphocyte ratio (NLR) values are well-known predictors of mortality [5]. Weng et al. [6] developed a new mortality predictor by adding age to these laboratory parameters and named this newly developed EWS as the age, NLR, D-dimer, CRP (ANDC) score. Other researchers [7,8] have discussed the ability of the ANDC score to predict mortality in different patient groups such as geriatrics or pediatrics. In this study, we aimed to test the ability of the ANDC score to predict prolonged hospitalization in SARS-CoV-2-infected patients.

Material and methods

Study design

This study was carried out as a prospective and observational study at a tertiary hospital with 685 beds, of which 152 were allocated to intensive care. During the peak periods of the pandemic, all the beds of the center where the study was conducted were reserved for patients with SARS-CoV-2.

Study population

Patients who applied to the hospital's emergency department with SARS-CoV-2 symptoms and findings between December 15, 2021, and March 15, 2022, and were hospitalized with positive rt-PCR results were included in the study. Patients who were admitted directly to the intensive care unit directly from emergency department or those who died at emergency department were excluded from the study because of the possible short length of stay despite the severity of the disease. Patients who were transferred to another hospital during their stay were also excluded. The total duration of stay in the wards and intensive care units was considered as the total length of hospital stay.

Data collection

Patients' demographics and comorbidities were recorded in the study form. The length of hospital stays and laboratory parameters were obtained from the hospital computer-based data recording system. Comorbidities were recorded as chronic obstructive pulmonary disease, hypertension, diabetes mellitus, coronary artery disease, congestive heart failure, asthma, history of malignancy, chronic kidney disease, and hyperlipidemia. As laboratory parameters, white blood cell count, neutrophil count, lymphocyte count, platelet count, hemoglobin count, hematocrit count, mean platelet volume, mean corpuscular volume, sodium, potassium, glucose, blood urea nitrogen, creatinine, albumin, alanine aminotransferase, aspartate aminotransferase, D-dimer, troponin ferritin, and CRP were recorded. NLR, platelet-to-lymphocyte ratio (PLR), and ANDC score were calculated. The ANDC score was calculated using the formula (1.14 x Age (years) - 20) + 1.63 x NLR + 5.00 x D-dimer (mg/L) + 0.14 x CRP (mg/L). Patients were divided into two groups as expected hospitalization and prolonged hospitalization according to their length of hospital stay. Patients who were hospitalized for seven days or longer were included in the prolonged hospitalization group. In-hospital mortality data were obtained from the hospital computer-based data recording system.

Statistical analysis

Jamovi software (The Jamovi Project, Version 1.6.21.0; 2020) was used for statistical analyses. The conformity of the parameters to the normal distribution was evaluated with the Shapiro-Wilk test. Categorical data were shown using number and percentages, and continuous data with median and 25th-75th percentile values. The chi-square test was used for the intergroup comparison of categorical data, and the Mann-Whitney U test for the intergroup comparison of continuous data. The receiver operating characteristic (ROC) analysis was performed to measure the ability of the parameters to predict prolonged hospitalization. The optimum cut-off levels for the parameters were found using Youden's index with the formula, sensitivity + (1 - specificity). The results of the ROC analysis were shown using the area under the curve (AUC), accuracy, positive predictive value, negative predictive value, 95% confidence interval, and cut-off value. The odds ratios were used to determine and compare the predictive ability of the parameters. P values below 0.05 were accepted as statistically significant.

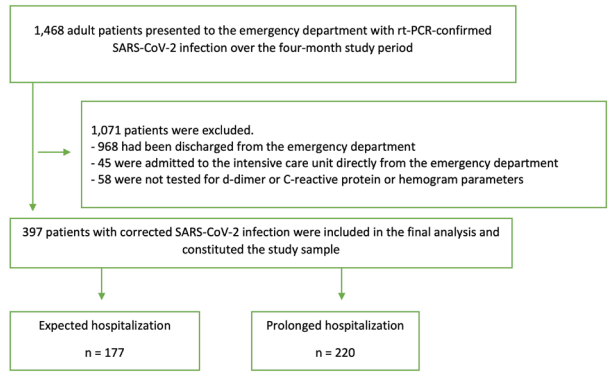
Ethics

Ethical approval for the study was obtained from the Republic of Turkey Ministry of Health Ümraniye Hospital Clinical Researches Ethical Committee (approval number: B.10.1.TKH.4.34.H.GP.0.01/320). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Consent to participate in the study was obtained from the patients with sufficient consciousness and from the relatives of those with impaired consciousness.

Results

A total of 1,468 patients presented to the emergency department with confirmed SARS-CoV-2 infection during the period from December 15, 2021 to March 15, 2022. Of those patients, 968 were excluded because they had been discharged from the emergency department, 45 because they were admitted to the intensive care unit directly from the emergency department, and 58 because they were not tested for D-dimer or CRP or hemogram parameters. The remaining 397 patients constituted the study sample, and their data were included in the final analysis (Figure 1).

Figure 1 - Flowchart of the study



Of the 397 patients in the sample, 209 (52.6%) were female. The median age was 74 years (25th-75th percentiles: 63-82), and their median length of hospital stay was 8 days (25th-75th percentiles: 5-13). The in-hospital mortality rate was 16.6% (66 patients).

The median of D-dimer, neutrophil count, lymphocyte count, NLR, and CRP values were found to be 830 (500-1930) mg/dL, 5.67 (3.93-7.75) 103/µL, 1 (0.66-1.38) 103/µL, 5.64 (3.37-9.38), and 74 (32.6-139) mg/dL, respectively. The most frequent comorbidity was hypertension (230 patients, 57.9%). The baseline characteristics and laboratory parameters of the enrolled patients are presented in Table 1.

Table 1 Baseline characteristics and laboratory parameters of the enrolled patients and their comparison between the expected and prolonged hospitalization groups

Variables	Total n = 397	Expected hospitalization n = 171 (41.3%)	Prolonged hospitalization n = 226 (56.9%)	P
	n (%)/median (25th-75th percentiles)	n (%)/median (25th-75th percentiles)	n (%)/median (25th-75th percentiles)	
Age	74 (63 - 82)	73 (59 - 81)	76 (67 - 83)	**0.014**
<65 years	105 (26.4%)	58 (33.9%)	47 (20.8%)	**0.003**
≥65 years	292 (73.6%)	113 (66.1%)	179 (79.2%)	
Gender				
Female	209 (52.6%)	96 (56.1%)	113 (50.0%)	0.225
Male	188 (47.4%)	75 (43.9%)	113 (50.0%)	
Symptoms				
Cough	141 (35.5%)	61 (35.7%)	80 (35.4%)	0.955
Shortness of breath	171 (43.1%)	64 (37.4%)	107 (47.3%)	**0.048**
Comorbidities				
Chronic obstructive pulmonary disease	51 (12.8%)	18 (10.5%)	33 (14.6%)	0.229
Hypertension	230 (57.9%)	92 (53.8%)	138 (61.1%)	0.147
Diabetes mellitus	140 (35.3%)	64 (37.4%)	76 (33.6%)	0.433
Coronary artery disease	90 (22.7%)	31 (18.1%)	59 (26.1%)	0.060
Congestive heart failure	43 (10.8%)	13 (7.6%)	30 (13.3%)	0.072
History of malignancy	47 (11.8%)	20 (11.7%)	27 (11.9%)	0.939
Hyperlipidemia	27 (6.8%)	7 (4.1%)	20 (8.8%)	0.062
Chronic kidney disease	40 (10.1%)	11 (6.4%)	29 (12.8%)	**0.036**
Vital parameters				
Systolic blood pressure	125.0 (112.0 - 142.0)	121.0 (110.0 - 140.0)	128.0 (115.0 - 144.0)	**0.014**
Diastolic blood pressure	72.0 (64.0 - 80.0)	70.0 (60.0 - 80.0)	73.0 (64.0 - 80.0)	0.122
Pulse pressure	85.0 (77.0 - 95.0)	85.0 (78.0 - 95.0)	84.0 (75.0 - 95.0)	0.147
Oxygen saturation	95.0 (93.0 - 97.0)	96.0 (94.0 - 97.0)	95.0 (92.0 - 97.0)	**0.002**
Laboratory parameters				
White blood cell count (103/μL)	7.21 (5.60 - 9.86)	7.09 (5.27 - 9.22)	7.43 (5.63 - 10.44)	0.203
Neutrophil count (103/μL)	5.67 (3.93 - 7.75)	5.30 (3.71 - 7.03)	5.92 (4.17 - 8.45)	0.071
Lymphocyte count (103/μL)	1.00 (.66 - 1.38)	1.06 (0.71 - 1.41)	0.96 (0.60 - 1.38)	0.178
Hemoglobin (g/dL)	12.2 (10.8 - 13.8)	12.4 (10.8 - 13.7)	12.1 (10.8 - 13.8)	0.934
Hematocrit (%)	37.1 (33.0 - 41.1)	37.1 (33.1 - 41.1)	37.1 (32.9 - 41.6)	0.875
Mean corpuscular volume (fL)	86.9 (82.9 - 91.0)	85.6 (82.9 - 90.9)	87.8 (82.8 - 91.2)	0.234
Platelet count (103/μL)	198.0 (156.0 - 256.0)	208.0 (164.0 - 270.0)	194.0 (146.0 - 241.0)	0.071
Mean platelet volume (fL)	9.8 (8.9 - 10.5)	9.6 (8.8 - 10.3)	10.0 (9.1 - 10.6)	**0.016**
Blood urea nitrogen (mg/dL)	43.6 (30.0 - 63.1)	38.4 (25.2 - 58.0)	46.7 (32.7 - 67.5)	**<0.001**
Creatinine (mg/dL)	.97 (.76 - 1.30)	0.88 (0.67 - 1.17)	1.04 (0.81 - 1.37)	**<0.001**
Sodium (mEq/L)	137.0 (134.5 - 139.5)	137.0 (134.5 - 139.0)	137.0 (134.5 - 140.0)	0.479
Potassium (mmol/L)	4.33 (3.99 - 4.68)	4.28 (3.99 - 4.66)	4.35 (4.00 - 4.69)	0.333
Albumin (g/dL)	36.00 (33.00 - 39.00)	36.01 (33.54 - 40.00)	35.86 (32.73 - 39.00)	0.060
Ferritin (mg/dL)	299.80 (144.90 - 589.35)	248.20 (127.00 - 492.70)	356.45 (181.30 - 700.20)	**0.013**
D-dimer (mg/dL)	830 (500 - 1930)	770 (470 - 1350)	895 (540 - 2060)	**0.008**
Troponin (cTnI) (ng/mL)	20.62 (9.74 - 38.84)	17.18 (7.72 - 26.97)	24.67 (11.42 - 56.03)	**<0.001**
Aspartate aminotransferase (IU/L)	19 (12 - 30)	18 (12 - 27)	19 (13 - 32)	0.183
Alanine aminotransferase (IU/L)	28 (21- 40)	26 (18 - 36)	30 (23 - 43)	**<0.001**
C-reactive protein. (mg/dL)	74.03 (32.59 - 138.70)	69.87 (27.55 - 120.39)	77.03 (40.89 - 149.38)	**0.024**
Glucose (mg/dL)	121 (101 - 163)	122.0 (102 - 155)	118.0 (100 - 167)	0.965
Neutrophil-to-lymphocyte ratio	5.64 (3.36 - 9.38)	4.89 (3.13 - 9.06)	6.17 (3.56 - 10.04)	**0.048**
Platelet-to-lymphocyte ratio	197.060 (130.682 - 310.170)	197.753 (133.047 - 296.610)	196.102 (127.273 - 336.000)	0.729
C-reactive protein-to-albumin ratio	2.09 (.86 - 3.92)	1.80 (0.70 - 3.24)	2.25 (1.05 - 4.25)	**0.014**
Blood urea nitrogen-to-/albumin ratio	1.24 (.81 - 1.80)	1.07 (0.70 - 1.59)	1.33 (0.90 - 1.99)	**<0.001**
Length of hospital stay (days)	7.6 (4.9 - 12.0)	4.6 (3.0 - 5.7)	11.1 (8.3 - 16.0)	**<0.001**
In-hospital mortality	66 (16.6%)	13 (7.6%)	53 (23.5%)	**<0.001**
ANDC	108.205 (87.649 - 136.891)	100.723 (78.983 - 129.253)	113.114 (94.495 - 140.546)	**<0.001**

The univariant analysis was performed to determine the differences in the investigated parameters between the study groups. Significant differences were found between the expected and prolong hospitalization groups in terms of the ANDC score (101 (80.1-127) versus 114 (94.3-141), $p < 0.001$), age (73 (59-81) versus 76 (67-83) years, $p = 0.014$), D-dimer (770 (480-1310) versus 895 (555-2075) mg/dL, $p = 0.008$), NLR (4.89 (3.13 - 9.06) versus 6.17 (3.56 - 10.04), $p = 0.048$), and CRP (69.87 (27.55-120.39) versus 77.03 (40.89-149.38) mg/L, $p = 0.024$). Table 1 shows the comparison of all the parameters between the two groups.

Table 2 Ability of the investigated parameters in to predict prolonged hospitalization in patients with SARS-CoV-2 infection

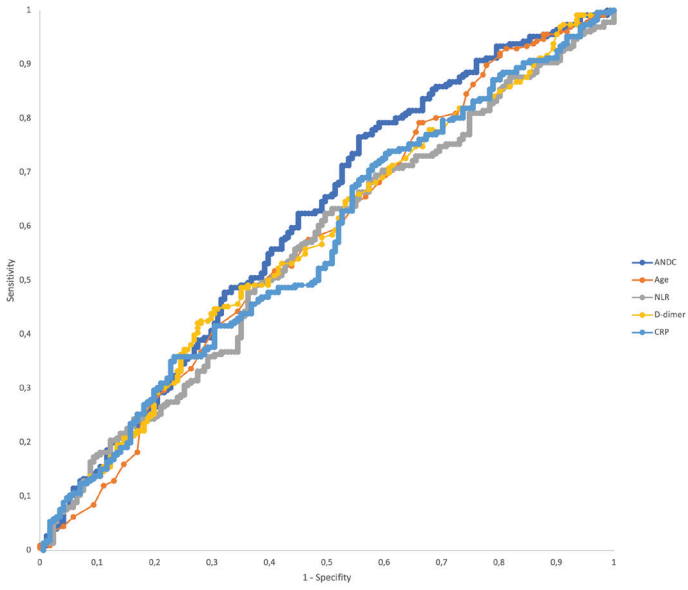
Variables	AUC	Cut-off value	95% CI	Accuracy	Sensitivity	Specificity	PPV	NPV	PLR	NLR	p value
ANDC	0.609	94.12	0.552-0.665	0.627	76.55%	44.44%	64.55%	58.91%	1.38	0.53	<0.001
Age	0.572	65	0.515-0.630	0.597	79.20%	33.93%	61.29%	55.21%	1.20	0.61	0.013
D-dimer	0.573	1190	0.516-0.630	0.552	42.04%	72.51%	66.90%	48.63%	1.53	0.80	0.012
Neutrophil-to-lymphocyte ratio	0.557	4.94	0.500-0.615	0.572	62.39%	50.29%	62.39%	50.29%	1.26	0.75	0.05
C-reactive protein	0.566	46.8	0.509-0.623	0.587	71.2%	42.09%	62.04%	52.62%	1.23	0.68	0.023

Table 3 Comparison of the area under the curve values of the investigated parameters according to the DeLong equality test

Variables		Age	D-dimer	Neutrophil-to-lymphocyte ratio	C-reactive protein
ANDC	AUC	0.609-0.572	0.609-0.573	0.609-0.557	0.609-0.566
	p value	0.206	0.284	0.08	0.025
Age	AUC		0.572-0.573	0.572-0.557	0.572-0.566
	p value		0.989	0.688	0.876
D-dimer	AUC			0.573-0.557	0.573-0.566
	p value			0.670	0.859
Neutrophil-to-lymphocyte ratio	AUC				0.557-0.566
	p value				0.796

The ROC analysis was performed to show the predictive power of the ANDC score and the parameters of ANDC in prolonged hospitalization. The AUC value of the ANDC score was found to be 0.609 and 75.91% sensitivity, 42.94% specificity, 62.3% positive predictive value, and 58.9% negative predictive value at a cut-off value of 93.5. The complete results of the ROC analysis are presented in Table 2 and Figure 2. The statistical differences in the AUC values according to the DeLong equality test are presented in Table 3.

Figure 2 - Receiver operating characteristic curve of the parameters for predicting prolonged hospitalization



The odds ratios of the investigated parameters were also calculated to compare their predictive ability in prolonged hospitalization. The odds ratios of the ANDC score, age, D-dimer, NLR, and CRP were calculated as 2.6, 1.9, 1.9, 1.6, and 1.8 respectively. The details of this analysis are presented in Table 4.

Table 4 Odds ratio results obtained according to the optimum cut-off values

Variables	Odds ratio	95% confidence interval
ANDC	2.611	1.697-4.018
Age	1.955	1.245-3.069
D-dimer	1.913	1.248-2.933
Neutrophil-to-lymphocyte ratio	1.678	1.122-2.511
C-reactive protein	1.801	1.185-2.737

Discussion

In this study, we investigated to ability of the ANDC score and the parameters used in its calculation to predict prolonged hospitalization in SARS-CoV-2-infected patients. According to the results, there was a statistically significant difference between the prolonged and expected hospitalization groups in terms of all the investigated parameters. The odds ratio of the ANDC score was greater than the odds ratios of each of the parameters used to calculate this score. To the best of our knowledge, this study is the first to investigate whether the ANDC score could predict prolonged hospitalization in SARS-CoV-2-infected patients.

With the increased burden on the health system due to the pandemic, scoring systems such as ANDC, the Pandemic Respiratory Infection Emergency System Triage (PRIEST) Severity Score and the COVID-19 Community Mortality Risk Prediction tool (CoCoMoRP) have been developed using methods such as machine learning to assess the severity of COVID-19 cases. These scoring studies are based on vital parameters, laboratory parameters, and supportive treatment [6, 9, 10]. In a study conducted with patients with COVID-19 in the early period of the pandemic, Weng et al. [11] used the least absolute shrinkage and selection operator (LASSO) regression analysis and identified independent variables as age, D-dimer, NLR, and CRP. The authors named the new model they created with the LASSO method as the ANDC score. The primary outcome in the study of Weng et al. was all-cause death. They performed the ROC analysis to test the ability of the ANDC score to predict this outcome. They reported the AUC value as 0.921 [6], which is close to an ideal predictor [12]. To test the model, they validated the score with patients from another

hospital and reported the model as successful. In another study, Bilge et al. [13] tested the ability of the ANDC score to predict mortality in patients with malignancy hospitalized due to SARS-CoV-2-associated pneumonia. They reported the AUC value as 0.69 (95% confidence interval: 0.54 - 0.84), and the sensitivity and specificity as 80% and 46%, respectively at the cut-off value of 100.

In the current study, first, we performed a univariant analysis to reveal the relationship between the ANDC score and prolonged hospitalization. We found that the group with prolonged hospitalization had a higher ANDC score. We performed the ROC analysis to assess the ability of the score to predict prolonged hospitalization. We determined that the ANDC score had a relatively low AUC in predicting prolonged hospitalization. In addition, we compared the AUC values of the ANDC score with those of the parameters constituting this score. In our analysis using the DeLong equality test, there was no statistically significant difference between the AUC values of the ANDC score and age and D-dimer. According to the results of this analysis, it can be concluded that the ANDC score is not better than age and D-dimer in predicting prolongation of hospital stay. Finally, we dichotomized the parameters according to the cut-off values obtained by the ROC analysis and calculated the odds ratios. We determined that the ANDC score had an odds ratio above the odds ratios of the parameters that made up this score.

The most important shortcoming of our study was the relatively limited sample size and single-center design, limiting the generalizability of the findings. Another important limitation is that SARS-CoV-2 subtypes were not studied. However, our study included patients infected with SARS-CoV-2 in the fourth peak period of the pandemic, during which the delta variant was dominant in the Northern hemisphere.

Conclusion

The present study was done to test the ability of the ANDC score, which is known to predict mortality in SARS-CoV-2 infected patients, to predict prolonged hospitalization in SARS-CoV-2 infected patients. Based on all the observations from the present study, it was concluded that the ANDC score is a predictor of prolonged hospitalization in SARS-CoV-2 infected hospitalized patients.

Besides, compared to using the age, D-dimer, NLR, and C-reactive protein values alone, ANDC was found to be more valuable in predicting prolonged hospitalization in SARS-CoV-2 infected hospitalized patients. On the other hand, we recommend confirming the results of our study with larger samples and multicenter studies.

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Investigation of the relationship between fear of COVID-19 and mother to infant bonding in postpartum women

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Abstract

Objective: The aim of the study is to evaluate the relationship between the fear of COVID-19 and mother to infant bonding in postpartum women.

Material and methods: This descriptive cross-sectional study was conducted online from social media platforms. The women who were in the postpartum period (between 1-40 days), using smart phones, and healthy for themselves and their babies were included in the study. The sample was determined by power analysis and the study was completed with 205 puerperal women. Personal Information Form, Coronavirus (COVID-19) Fear Scale and Mother to Infant Bonding Scale (MIBS) were used to collect data.

Results: It was determined that the mean score of the Women's Fear of Coronavirus (COVID-19) Scale was 16.85±6.42 and the mean score of the Mother to Infant Bonding Scale was 3.18±3.58. It was found that there was no significant relationship between fear of coronavirus and mother to infant bonding levels of the women participating in the study (r=0.046, p=0.478). It has been observed that the income status of women, regular doctor check-ups, having a coronavirus disease, being vaccinated against COVID-19 affect the fear of COVID-19, while mother to infant bonding is affected by regular doctor check-ups, being vaccinated against COVID-19, and losing their family due to COVID-19.

Conclusion: It was concluded that women in the postpartum period should be supported by health professionals from the pregnancy period in order to cope with the fear of COVID-19 and to achieve safe and healthy mother to infant bonding.

Key words: fear of coronavirus, mother to infant bonding, postpartum period

Introduction

While it has been reported that those with chronic diseases are at risk for coronavirus transmission and complications during the pandemic process [1], it has also been stated that healthy pregnant women will be more affected by coronavirus disease (COVID-19) due to their immune responses. In addition to pregnancy, new mothers and their babies have also been seriously affected during the pandemic process. Approaches in the delivery process of pregnant women with maternal COVID-19 have changed [2,3,4]. Decreased social support to maintain social distance in the postpartum period [5], and disagreements on the appropriateness of mother and baby staying in the same room [6,7], have brought along many important problems. One of these problems is the interruption of mother to infant bonding.

The concept of bonding is defined as the bond formed between the baby and the person who cares for

the baby in the first degree, which develops and strengthens the baby's sense of trust [8]. The concept of mother to infant bonding is a type of bond that starts with the movements and development of the baby during pregnancy and is expected to continue by getting stronger [9]. Factors affecting mother-infant bonding attachment are extremely effective in the development and strengthening of the role of motherhood in women [10].

The pandemic period, which left humanity with many problems in every sense, also caused the bonding of mother and baby in the postpartum period to be negatively affected. There are opinions that staying in the same room with mothers who are sick or in contact with COVID-19 may increase the risk of transmission [6,7,11]. However, although the evidence is insufficient, the isolation measures applied negatively affect the breastfeeding process, maternal and neonatal health by preventing mother-infant bonding [12,13].

Mother to infant bonding has been affected by many factors during the pandemic process. One of these reasons is the problems experienced with breastfeeding [13]. Because breastfeeding is the period that has the most important role in the initiation and strengthening of the bond between the mother and the baby. According to the report of the Centers for Disease Control and Prevention (CDCP), it is reported that breastfeeding is not a transmission route for the infant for COVID-19 and all other respiratory tract infections [14]. However, a clear conclusion could not be reached due to insufficient research results. In the study conducted by Cojocaru et al., 1989 pregnant women were screened for COVID-19. According to the screening results, 86 pregnant women were found to have COVID-19 patients. It was stated that 14 of 31 pregnant women were separated from their babies, and skin-to-skin contact and breastfeeding were provided in 17 of them. It has been stated that the COVID-19 test result for all newborns was negative [15]. This shows that COVID-19 disease is not an obstacle for mother to infant bonding. In addition, in different studies in the literature [16,17], there were no clinical findings suggestive of COVID-19 in newborns born to mothers with COVID-19, and SARS-CoV-2 it has been shown to be negative. In a multicenter cohort study conducted in Turkey, babies of mothers with COVID-19 were evaluated and reverse transcription polymerase chain reaction (RT-PCR) results were found to be positive in 4 out of 120 newborns. In addition, breastfeeding status of newborns was interrupted in this study. It has been emphasized that this may be due to the inadequacy of family support, fear and lack of knowledge [12].

There is limited information on the evaluation of the relationship between fear of COVID-19 and mother to infant bonding in mothers who have given birth during the COVID-19 pandemic. Evaluation of mother to infant bonding during the pandemic period is also important in terms of gaining healthy motherhood experience and raising healthy generations. The aim of the research conducted in this direction; To evaluate the relationship between fear of COVID-19 and mother to infant bonding in postpartum women.

Material and methods

Research design, population and sample

The research was designed in descriptive cross-sectional type. Between May-July 2022, women in the 18-49 age group were reached from social media platforms (WhatsApp, Twitter, Instagram, Facebook, Pinterest and Snapchat, etc.) and all postpartum (between 1-40 days) women were reached between the specified dates has created. In the study of Engin and Kuzlu Ayyıldız (2021) (n=368) in which the factors affecting mother to infant bonding were evaluated to determine the sample size at the beginning of the study, the baby's desire status was considered as a common parameter [18]. As a result of the analysis based on the Type I error 0.01, Type II error 0.01 (power 0.99), the sample size was calculated as 205 with an effect size of 0.3. According to the results obtained from the research (n=235) G-Power 3.1.9.4. When the posthoc power analysis was performed in the program, the effect size was found to be 0.22. In the power analysis based on the confidence interval of 95%, the significance level of 0.05 and the effect size of 0.22, the power of the research was calculated as 93% and was considered sufficient.

As sampling inclusion criteria, women aged 18 and over, those with primigravida and multigravida, those who had vaginal or cesarean delivery, those who were in the postpartum period (between 1-40 days), those who had at least primary school education, and those who had the capacity to read and understand Turkish, smart phone users and those who voluntarily participated in the research. Those who did not have their baby with them (in the neonatal intensive care unit), those who were diagnosed with postpartum depression, and those who had a psychiatric diagnosis and used medication were excluded from the sample.

Data collection tools

Personal information form, Coronavirus (COVID-19) Fear Scale and Mother to Infant Bonding Scale were used to collect data.

Personal information form: It consists of questions about socio-demographic and obstetric characteristics. It includes questions such as the age of the woman, education level, employment status, family type, income level, desired pregnancy status, baby's gender, type of birth, and problems at birth. In addition, it is included in the questions about the situation of having COVID-19 and being vaccinated.

Coronavirus (COVID-19) Fear Scale: Developed by Ahorsu et al. (2020) [19]. The scale has a 5-point Likert-type rating system (7 items) (1: Strongly disagree and 5: Strongly agree). The scores that can be obtained from the scale range from 7 to 35. A high score from the scale means experiencing a high level of fear of coronavirus. The scale was adapted to Turkish by Bakioğlu et al. in 2020 [20]. It has been determined that the scale is a psychometric tool to be used to evaluate fears of COVID-19 among individuals of both genders and all age groups. In the present study conducted with women in the postpartum, the Cronbach Alpha coefficient was calculated as 0.85.

Mother to Infant Bonding Scale (MIBS): The scale can be applied from the first day after birth and the feelings of the mother towards her baby can be expressed with a single word. This scale, which can be filled in by the mother or father, shows the relationship between the bond established between the mother/father and the infant and the first period mood. The original name is "Mother-to-Infant Bonding Scale" and was developed by Taylor et al. (2005) [21]. It was adapted into Turkish by Aydemir and Alparslan (2009) [22]. MIBS is a 4-point Likert scale consisting of 8 items. The answers consisting of four options are scored between 0-3, the lowest score that can be obtained from the scale is 0 and the highest score is 24. Positive and negative emotions are scored in reverse. In the evaluation, the 1st, 4th, and 6th items are positive emotion expressions and are scored as 0,1,2,3; 2.,3.,5.,7. Items 8 and 8 are negative emotional expressions and are scored as 3,2,1,0 and reversely. An increase in the total score indicates worse bonding. The Cronbach Alpha value of the scale was reported to be 0.66 [22]. According to the evaluation made in our research, the Cronbach Alpha value was calculated as 0.70.

Ethical Approval

Written and verbal consent was obtained from all women participating in the study. Approval was obtained from the Ethics Committee of the Faculty of Health Sciences of Karamanoğlu Mehmetbey University (Date: 30.03.2022, No: 02-2022/11).

Data analysis

Statistical analyzes were performed with the Statistical Package for Social Sciences (SPSS) 22.0 statistical package program. Number, percentage, mean and standard deviation were calculated for descriptive statistics. Kolmogrow-Smirnov test, skewness and kurtosis coefficients were examined to determine whether the scale scores were normally distributed, and it was determined that the distribution was not normal. Therefore, non-parametric tests were used. The Spearman correlation test was used to evaluate the relationship between the fear of COVID-19 and the Mother to Infant Bonding Scale (MIBS) score averages of the women participating in the study. When comparing the mean scores of the Mother to Infant Bonding Scale (MIBS) with the socio-demographic, obstetric and COVID-19 related information, independent groups were compared with the Mann Whitney U test, while comparing the mean scores of more than two groups, Kruskal Wallis test and age, number of births and number of postpartum days were used. Spearman correlation test was used to evaluate the relationships. The significance level of the findings obtained from the study was evaluated at the 95% confidence interval and at the $p<0.05$ significance level.

Results

The mean age of women is 28.29±5.10 years. The majority of the participants (35.7%) have secondary school education. It is seen that 79.1% of the women do not work in a job that generates income and the income of the majority (66.0%) is equal to their expenses (Table 1). When the obstetric characteristics of the participants were evaluated, it was determined that 88.9% of the women wanted pregnancy. It was determined that 95.3% of them had regular doctor checks during pregnancy. 41.3% of the women had a normal (vaginal) delivery. It was determined that the majority of women who experienced complications during the delivery process had a decrease in amniotic fluid (n=4, 1.7%). Other women were found to experience fetal distress (n=4, 1.7%), cord entanglement (n=3, 1.3%), and non-progressive delivery (n=2, 0.9%). It was determined that most of them (51.9%) needed support for breastfeeding after delivery. 59.6% of the women received the COVID-19 vaccine. It was determined that 53.7% (n=72) of these women had the COVID-19 vaccine (Table 1).

Table 1 Socio-Demographic, Obstetric and COVID-19-Related Characteristics of Women (n=235)

Characteristics	Average	SD
Age	28.29	5.10
	n	**%**
Educational status		
Primary school	27	11.5
Middle School	84	35.7
High school	64	27.2
Bachelor and above	60	25.5
Working status		
Yes	49	20.9
No	186	79.1
Economic condition		
Low	58	24.7
Middle	155	66.0
High	22	9.4
Family Type		
Nuclear family	189	80.4
Extended family	46	19.6
Desired state of pregnancy		
Yes	209	88.9
No	26	11.1
	Avarage	**SD**
Number of births	1.97	0.96
Number of abortion/curettage	0.38	0.65
	n	**%**
Baby's gender		
Girl	124	52.8
Boy	111	47.2
Regular attendance at prenatal checkups		
Yes	224	95.3
No	11	4.7
Type of birth		
Normal (vaginal) birth	97	41.3
Cesarean delivery	138	58.7
Complications at birth		
Yes	16	6.8
No	219	93.2
Postpartum care needed		
Breast-feeding	122	51.9
Mobilization	75	31.9
Personal hygiene	24	10.2
Baby care	14	6.0
	Avarage	**SD**
COVID-19 status		
Yes	134	57.0
No	101	43.0
Getting a COVID-19 vaccine		
Yes	140	59.6
No	95	40.4
Status of having COVID-19 in the family		
Yes	209	88.9
No	26	11.1
Death in the family due to COVID-19		
Yes	13	94.5
No	222	5.5

Table 3 The relationship between the mean scores of the Fear of Coronavirus (COVID-19) Scale and the Mother to Infant Bonding Scale (n=235)

	Fear of Coronavirus (COVID-19) Scale	
	r*	p
Mother to Infant Bonding Scale	0.046	0.478

*Spearman corelation test

Table 2 Mean scores and median distribution of Women's Postpartum Fear of Coronavirus (COVID-19) Scale and Mother to Infant Bonding Scale (n=235)

Mean±SD*	Median	(Minimum-Maximum)	Cronbach alpha
Fear of Coronavirus (COVID-19) Scale	16.85±6.42	16.00 (7.00-35.00)	0.85
Mother to Infant Bonding Scale (MIBS)	3.18±3.58	2.00 (0.00-15.00)	0.70

*SD: Standard deviation

Table 4 Factors Affecting the Mean Scores of the Fear of Coronavirus (COVID-19) Scale by Women's Socio-Demographic, Obstetrical Characteristics and Knowledge about COVID-19

Fear of Coronavirus (COVID-19) Scale		
Characteristics		
Age		r*=-0.013, p=0.843
		Mean±SD**
Educational status	Primary school	17.518±6.45
	Middle School	17.357±6.14
	High school	16.015±6.99
	Bachelor and above	16.750±6.24
		KW=2.685, p=0.443
Working status	Yes	16.000±5.99
	No	17.080±6.53
		U=4110.000, p=0.290
Economic condition	Low	18.086±7.01
	Middle	16.780±6.28
	High	14.136±4.98
		KW=5.504, p=0.049* **
Family Type	Nuclear family	16.963±6.49
	Extended family	16.413±6.21
		U=4112.000, p=0.569
Desired state of pregnancy	Yes	16.861±6.46
	No	16.807±6.20
		U=2660.000, p=0.861
Number of births		r=0.070, p=0.282
Number of abortion/ curettage		r=0.073, p=0.263
Regular attendance at prenatal checkups	Yes	15.588±5.88
	No	18.270±6.73
		U=5248.500, p=0.002* **
Type of birth	Normal (vaginal) birth	16.360±6.73
	Cesarean delivery	17.209±6.20
		U=6168.000, p=0.305
Complications at birth	Yes	15.812±6.42
	No	16.931±6.43
		U=1647.000, p=0.689
Postpartum care needed	Breast-feeding	16.516±6.27
	Mobilization	16.813±6.71
	Personal hygiene	17.208±5.95
	Baby care	19.428±6.96
		KW=2.673, p=0.445
COVID-19 status	Yes	16.134±6.32
	No	17.811±6.47
		U=5714.500, p=0.041* **
Getting a COVID-19 vaccine	Yes	17.771±6.38
	No	15.505±6.28
		U=5331.500, p=0.010* **
Status of having COVID-19 in the family	Yes	17.148±6.42
	No	14.500±6.05
		U=2095.000, p=0.057
Death in the family due to COVID-19	Yes	19.307±5.61
	No	16.711±6.45
		U=1053.000, p=0.101

*Spearman corelation test, **SD: Standard deviation, *** p<0.05

Table 5 Factors Affecting the Mean Mother to Infant Bonding Scale Scores According to Women's Socio-Demographic, Obstetrical Characteristics and Knowledge about COVID-19 (n=235)

Mother to Infant Bonding Scale		
Characteristics		
Age		r*=0.030, p=0.650
		Mean±SD*
Educational status	Primary school	3.925±3.80
	Middle School	2.690±3.28
	High school	3.062±3.56
	Bachelor and above	3.666±3.87
		KW=4.961, p=0.175
Working status	Yes	3.224±3.50
	No	3.172±3.61
		U=4424.500, p=0.750
Economic condition	Low	2.965±3.59
	Middle	3.258±3.58
	High	3.227±3.74
		KW=0.887, p=0.642
Family Type	Nuclear family	2.989±3.40
	Extended family	3.978±4.19
		U=3916.000, p=0.289
Desired state of pregnancy	Yes	3.090±3.60
	No	3.923±3.44
		U=2282.000, p=0.176
Number of births		r=-0.032, p=0.631
Number of abortion/ curettage		r=-0.065, p=0.324
Regular attendance at prenatal checkups	Yes	3.120±3.59
	No	4.454±3.20
		U=865.500, p=0.046***
Type of birth	Normal (vaginal) birth	2.793±3.18
	Cesarean delivery	3.360±3.64
		U=5005.000, p=0.476
Complications at birth	Yes	3.437±2.78
	No	3.164±3.64
		U=1541.500, p=0.414
Postpartum care needed	Breast-feeding	3.082±3.63
	Mobilization	3.666±3.89
	Personal hygiene	2.416±2.26
	Baby care	2.785±3.28
		KW=1.171, p=0.760
COVID-19 status	Yes	3.194±3.80
	No	3.168±3.29
		U=6409.500, p=0.481
Getting a COVID-19 vaccine	Yes	3.500±3.67
	No	5.715±3.41
		U=5689.000, p=0.049***
Status of having COVID-19 in the family	Yes	3.220±3.63
	No	2.884±3.24
		U=2586.000, p=0.683
Death in the family due to COVID-19	Yes	4.846±3.97
	No	3.085±3.54
		U=987.500, p=0.048***

*Spearman corelation test, **SD: Standard Deviation, *** p<0.05

It was determined that the mean score of the Women's Fear of Coronavirus (COVID-19) Scale was 16.85±6.42 and the mean score of the Mother to Infant Bonding Scale (MIBS) was 3.18±3.58 (Table 2).

It was determined that there was no significant relationship between fear of coronavirus and mother to infant bonding levels of the women participating in the study (r=0.046, p=0.478) (Table 3).

When the factors affecting the average score of the Fear of Coronavirus (COVID-19) Scale according to the socio-demographic characteristics of women were evaluated, it was determined that age, education status, employment status and family type did not affect the fear of coronavirus. However, it has been determined that income status has an effect on fear of coronavirus. Further analysis revealed that the significant difference between income status and fear of coronavirus stemmed from the difference between groups with income less than expenses and income more than expenses (U435.500, p=0.029) (Table 4).

It was determined that those who had regular doctor check-ups during pregnancy had a lower level of fear of coronavirus (U=5248.500, p=0.002) than women who did not. It was determined that the women who did not get the coronavirus disease and those who had the COVID-19 vaccine had a higher score on the Coronavirus (COVID-19) Fear Scale (Table 4).

It was determined that the socio-demographic and obstetric characteristics of the women participating in the study had no effect on mother to infant bonding. However, it was determined that mother to infant bonding (U=865.500, p=0.046) was better in women who had regular doctor check-ups during pregnancy. It was determined that women who had the COVID-19 vaccine had better mother to infant bonding (U=5689.000, p=0.049) than women who did not. As a surprising finding, it was determined that the mother to infant bonding of women who lost their family due to COVID-19 was worse (U=987.500, p=0.048) than women who did not (Table 5).

Discussion

In the study, it was determined that women's COVID-19 fear mean score was 16.85 point (SD=6.42) and mother-infant bonding scale mean score was 3.18 (SD=3.58) point. It was determined that the women's fear of COVID-19 was moderate and mother-infant bonding was in good condition. It was found that there was no significant relationship between fear of coronavirus and mother to infant bonding levels. Women in the postpartum period experience greater anxiety, stress and fear, as they consider the health of the newborn, which they are responsible for raising, breastfeeding and protecting, as well as their own health during the pandemic process [23]. Mother to infant bonding is a special relationship that develops over time and is important for mother and baby health. In the successful continuation of this relationship; Many factors such as health status of mother and baby, number of pregnancies, relationship status between parents, family ties, cultural structure, planned pregnancy, postpartum depression, socio-economic status, risky pregnancies are effective [24]. In the study, it was observed that although the women's COVID-19 fear levels were moderate, it was not an effective factor in mother-infant bonding. Celik et al. (2022), it was found that maternal function was higher than the average and even higher than the studies conducted before the pandemic. It has been concluded that during the pandemic, mothers act more sensitively and attentively to fulfill their maternal function under the threat of COVID-19, and thus they may be better adapted to maternal function [25]. Similarly, the good condition of mother-infant bonding in our study may have been caused by the fact that women were better adapted to maternal function during the pandemic.

It has been observed that the fear of coronavirus is higher among those with low income than those with equal and higher income. In the study of Stojanov et al. (2020), it was determined that postpartum depression and anxiety were higher in women with low income during the COVID-19 pandemic period [26]. The long duration of the pandemic has also led to economic problems such as staying at home and job loss. In addition to these, catching the disease may also cause an additional financial burden, which may have caused the fear of coronavirus to be more in these women.

It has been determined that those who have regular doctor check-ups during pregnancy have less fear of coronavirus than women who do not. Mızrak Şahin and Kabakçı (2021) and Demirel Bozkurt et al. (2022), it was observed that pregnant women who easily contacted their doctors, midwives and nurses during the pandemic and received telephone or online support were less anxious and felt more comfortable [27,28]. Our study result is compatible with the literature. The level of fear of coronavirus may have decreased as women who are informed about their own and their baby's condition both relax and become more conscious and take the necessary precautions against COVID-19. In the study, it was determined that the mother-baby bonding of women who had regular doctor check-ups during pregnancy was better. In the study of Bilgin and Alpar (2018), it was determined that there is a relationship between strong mother to infant bonding during pregnancy and receiving prenatal care [29]. Similarly, in our study, having information about the health status of their babies may have affected the mother to infant bonding positively by providing relief for mothers.

In the study, it was determined that those who did not get coronavirus disease and women who had the COVID-19 vaccine had a higher level of fear of coronavirus. The most effective method of protection against COVID-19 is COVID-19 vaccines. COVID-19 vaccines are safe and effective in pregnant and lactating women [30]. It is natural for women with a high fear of COVID-19 to be vaccinated to protect themselves from COVID-19 because of this fear. In the study of Kalafatoğlu and Yam (2021), it was seen that the COVID-19 fear levels of the participants who were not diagnosed with COVID-19 were higher than those who received it [31]. As uncertainty about the effects of the disease decreases in women who have had COVID-19 disease, it can be thought that their fears about COVID-19 are less.

It was determined that women who had the COVID-19 vaccine had better mother to infant bonding than women who did not. In particular, the fact that pregnant and postpartum women feel safe by getting vaccinated to protect themselves and their babies from COVID-19 may have enabled them to adapt better to their babies.

In the study, it was determined that women who lost their families due to COVID-19 had worse mother to infant bonding than women who did not. Özşahin and Aksoy (2020) found that individuals who were diagnosed with COVID-19 in their relatives experienced significantly higher levels of COVID-19 anxiety [32]. In another study, it was found that losing a loved one due to COVID-19 causes symptoms of post-traumatic stress, depression, perceived stress and insomnia in individuals [33]. Especially the loss of relatives diagnosed with COVID-19 may have affected women psychologically negatively, and may have had an impact on mother-infant bonding as a negative factor.

Conclusion

As a result, it was determined that the fear of COVID-19 in postpartum women was moderate and mother to infant bonding was in good condition, and there was no significant relationship between fear of coronavirus and mother to infant bonding levels. In addition, it has been observed that the income status of women, having regular doctor check-ups, having a coronavirus disease, being vaccinated for COVID-19 affect the fear of COVID-19, while mother-baby bonding is affected by regular doctor check-ups, being vaccinated against COVID-19 and experiencing loss in her family due to COVID-19. The conducted this study determined the factors that should be evaluated in order to minimize the affecting factors mother-infant bonding in pandemic situations that may be encountered now and in the future. It is believed that knowing the factors that may affect mother-infant bonding in pandemic situations that may affect the whole world in the long term will be beneficial to prevention in the early period. It is recommended that women be supported by health professionals starting from the pregnancy period in order to cope with the fear of COVID-19 and to achieve safe and healthy mother to infant bonding, group or individual trainings, early detection of bonding and elimination of problems.

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Circulating levels of Bcl-2 and its expression in the nasal mucosa of patients with chronic rhinosinusitis

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Abstract

Aim: To evaluate expression of anti-apoptotic Bcl-2 protein in the nasal tissue and its levels in blood serum of patients with chronic rhinosinusitis with (CRSwNP) and without nasal polyps (CRSsNP).

Material and methods: Expression of Bcl-2 in the sinonasal tissue and its levels in blood serum of patients with CRSsNP and CRSwNP were evaluated immunohistochemically and using ELISA, respectively.

Results: In patients with CRSsNP, Bcl-2 was overexpressed in nasal epithelial cells mainly in the atrophic regions. However, its upregulation was also observed in regions with epithelial cell proliferation. Immunostaining for Bcl-2 was stronger both in the stroma and epithelial lining compared with control subjects. The level of Bcl-2 in blood serum was elevated in both forms of chronic rhinosinusitis with a more pronounced increase in CRSwNP.

Conclusion: CRSsNP and especially CRSwNP are associated with overexpression of anti-apoptotic Bcl-2 in nasal epithelial cells and in the lamina propria against the background of elevated circulating concentrations of Bcl-2.

Key words: nasal polyps, nasal epithelial cells, apoptosis, B-cell lymphoma 2

Introduction

Chronic rhinosinusitis (CRS) is an inflammation of the nasal tissue and paranasal sinuses lasting for at least 12 consecutive weeks and affecting up to 10-12% of population [1,2]. This debilitating disease significantly affects the quality of life and is characterized by enormous economic costs for the healthcare system. Morphologically, two forms of CRS have been reported. Chronic rhinosinusitis with nasal polyps (CRSwNP) is accompanied by the development of noncancerous soft outgrowths in the nasal and paranasal tissue. They are referred to as nasal polyps. Another form is called chronic rhinosinusitis without nasal polyps (CRSsNP). In its case, sinonasal inflammation occurs without the formation of polyps [3,4]. Despite numerous hypotheses offered to explain the etiopathogenesis of both forms of the disease, it still remains not fully elucidated. However, converging lines of evidence demonstrate that some factors contribute to the development of both CRSwNP and CRSsNP: genetic predisposition, defects of the innate immunity, features of sinonasal microbiota, epigenetic factors, abnormal mucociliary clearance, etc. [5-7]. Although both subtypes of CRS share common characteristics, CRSwNP and CRSsNP have numerous distinct features [8]. In particular, inflammation in CRSwNP has been reported to be Th2-mediated, whereas in CRSsNP Th1 cell subset prevails [9]. The search for dissimilarities between CRSsNP and CRSwNP may help find novel targets for therapeutic interventions specific for a particular subtype of CRS.

It has been reported that cell death is of crucial importance for the regulation of any kind of inflammation, including the sinonasal one [10]. One of cell death modes is apoptosis, which occurs in response to a wide variety of stimuli. However, pro-apoptotic signaling pathways are counterbalanced by anti-apoptotic signaling, and B-cell lymphoma 2 (Bcl-2) protein is a key anti-apoptotic regulator in the cells [11]. It provides pro-survival signaling by inhibiting the intrinsic mitochondrial apoptotic pathway via regulating mitochondrial outer membrane permeabilization (MOMP), which is believed to be a point-of-no-return in the intrinsic pathway [12,13]. MOMP results in the release of cytochrome c from mitochondria, followed

by the formation of apoptosome and activation of caspases [14]. Since Bcl-2 provides selective survival advantage for cells, it is of huge importance for neoplasms. It has been reported that overexpression of Bcl-2 is observed in a wide range of tumors and premalignant lesions, which allows suggesting its important role at early stages of tumorogenesis [15,16].

Features of Bcl-2 expression in cells of sinonasal mucosa and the role of this anti-apoptotic protein in CRS have been under investigation with some promising results reported recently [17]. We have hypothesized that Bcl-2 expression may be altered in CRS. Exploration of Bcl-2 involvement in the pathogenesis of CRS may contribute to the emergence of Bcl-2-targeted therapy. Thus, our study may shed extra light on the pathogenesis of CRS and to broaden our knowledge about the differences between CRSwNP and CRSsNP.

The aim of the research was to assess and compare circulating levels of Bcl-2 and features of its expression in the nasal mucosa of patients with CRSsNP and CRSwNP.

Material and methods

Patients

A total of 30 patients with CRS were recruited from the Kharkiv Regional Clinical Hospital (Kharkiv, Ukraine). Fifteen of them were diagnosed with CRSsNP (9 males and 6 females). Their age ranged from 21 to 60 years with the mean age of 37.44±4.21 years. In other 15 patients, the diagnosis of CRSwNP was verified (8 males and 7 females). Their age varied from 20 to 58 years of age. They mean age reached 34.98±3.41 years. The control group consisted of 15 healthy subjects (7 males and 8 females) of 19 to 53 years of age. These patients underwent a correction of deviated septum (septoplasty) under combined general and regional anesthesia. The mean age of this group was 31.85±4.02 years. The control subjects did not show any clinical signs of inflammation in the upper airways. ‘‘EPOS 2012: European Position Paper on Rhinosinusitis and NPs 2012” guidelines were used to verify the diagnosis [18].

The exclusion criteria were smoking, acute or exacerbation of chronic inflammatory diseases, cystic fibrosis, allergic diseases and asthma, administration of glucocorticoids at least 1 month prior to the collection of biological materials, pregnancy, and endocrine diseases.

Collection of blood samples and determination of circulating levels of Bcl-2 by ELISA

In order to assess the circulating level of Bcl-2, we collected blood samples immediately after hospitalization. Blood was used to prepare serum by centrifugation. Bcl-2 values in blood serum were determined by ELISA test using Human Bcl-2 Platinum ELISA Kits (eBioscience, Austria). The level of Bcl-2 was expressed in ng/ml.

Immunohistochemical study of Bcl-2 expression in the nasal tissue

Nasal tissue samples were obtained exclusively during surgery if there were indications for it. Briefly, formalin-fixed and paraffin-embedded tissue samples were obtained and used for immunohistochemical evaluation of Bcl-2 expression. Four-µm-thick sections of nasal tissues were immunostained with anti-Bcl-2 antibodies (Thermo Fischer Scientific, UK). Staining was completed with 3,3′-Diaminobenzidine (DAB) with the formation of brown coloration indicating Bcl-2 expression.

Bioethics

The revised Helsinki Declaration (2000) and “Bioethical Expertise of Preclinical and Other Scientific Researches Conducted on Animals” (Kyiv, 2006) were strictly followed. The experiment protocol was approved by the Ethical Committee of Kharkiv National Medical University (minutes No 6 dated June 6, 2018). Informed consent was carefully read and signed by all patients and control subjects enrolled in this study.

Statistical analysis

Numerical values of circulating Bcl-2 levels were analyzed with the help of the non-parametric Kruskal-Wallis test, since three unmatched groups were compared. As a post-hoc test, we used the Dunn’s multiple comparison test. Data are represented as the medians and interquartile ranges. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad software, USA).

Results

Analysis of Bcl-2 expression in the nasal mucosa of control subjects showed that its expression was observed in some cells of the lamina propria and submucosa. Bcl-2 expression was also revealed in some nasal epithelial cells (NECs).

Figure 1 - Immunostaining for Bcl-2 in the nasal mucosa of a control individual. Brown staining shows Bcl-2 expression (red arrows). Some Bcl-2 positive cells are found in the lamina propria. 400x.

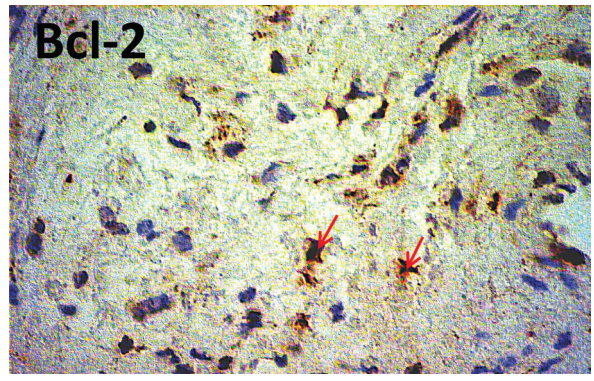


Figure 2 - Immunostaining for Bcl-2 in patients with CRSsNP. Strong staining is found in nasal epithelial cells. The number of Bcl-2-labelled nasal epitheliocytes is much higher compared with the control group (A, C, D; yellow arrows). Atrophic regions were characterized with a lower number of Bcl-2 positive cells in the lamina propria (D; red arrows). Bcl-2 expression was week in the glandular epithelial cells in regions with no signs of damage (B). In some regions, extracellular matrix cells strongly expressed Bcl-2 (B; red arrows). Figures 2a, 2c, 2d - 100x; Figure 2b – 400x.

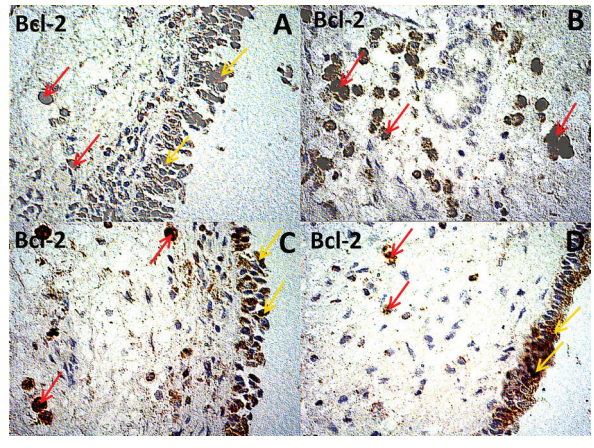


Figure 3 - Immunostaining for Bcl-2 in patients with CRSwNP. The amount of Bcl-2-positive cells in the lamina propria is visually higher than in the control group (A, B; red arrows). Strong Bcl-2 expression is observed in nasal epithelial cells that cover the polyps (B, C, D; yellow arrows). 400x

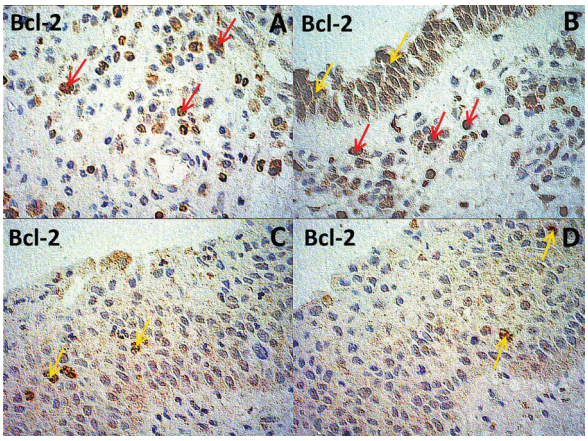
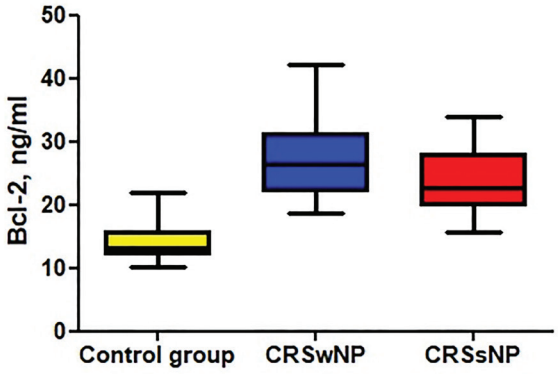


Figure 4 - Circulating levels of Bcl-2 in patients with CRSwNP and CRSsNP. Both forms of chronic rhinosinusitis are associated with statistically significant elevation of Bcl-2 in blood serum compared with healthy controls. Circulating Bcl-2 concentration increases 1.7–fold in CRSsNP ($p < 0.0001$), while in patients with CRSwNP its level is twice higher ($p < 0.0001$) compared with the control group. The statistical difference between Bcl-2 levels in blood serum in CRSsNP and CRSwP was insignificant ($p > 0.05$).



The mucosa of patients with CRSsNP was characterized by the presence of regions with either atrophic or regenerating epithelia. It is important to note that in the latter case the epithelial layer had several rows of NECs. This fact is of huge importance, since Bcl-2 was found to be significantly upregulated in NECs in atrophy. No or weak Bcl-2 expression was detected in the mucosal glandular epithelial cells in regions with no signs of damage. However, cells of the extracellular matrix (probably fibroblasts) were characterized by strong Bcl-2 expression.

NECs that cover polyps in patients with CRSwNP significantly express Bcl-2, excluding regions where excessive proliferation of NECs was observed. Under the epithelial layer in the extracellular matrix a higher number of Bcl-2-positive cells were found compared with the control group. They were either oval-shaped or spherical, i.e. these could be both fibroblasts and macrophages. Bcl-2 was overexpressed both in the lamina propria and NECs in patients with CRSwNP compared with control subjects.

The development of CRSwNP is accompanied by a more pronounced Bcl-2 expression in the epithelial layer that covers polyps and in their stroma than in the inflamed nasal mucosa of patients with CRSsNP.

All patients and control subjects were evaluated for Bcl-2 circulating concentrations on admission to the hospital. Serum levels of Bcl-2 in patients with both forms of CRS were found to be statistically significant ($p < 0.0001$) elevated compared with the control group (Figure 4). It is important to note that serum concentrations of Bcl-2 were twice elevated in CRSwNP compared with control subjects (26.33 [22.17; 31.12] ng / ml against 13.23 [12.14; 15.67] ng / ml in controls), while in patients with CRSsNP (22.67 [19.89; 27.90] ng / ml) this parameter was only 1.7-fold higher than in the control group. However, statistical analysis showed no significant difference ($p > 0.05$) between circulating Bcl-2 levels in patients with different morphological forms of CRS.

Discussion

It has been reported that NECs play an important role in inflammatory responses, which is not restricted to their passive barrier function. In addition to their barrier role, NECs release cytokines [19]. Thus, these cells are directly involved in the regulation of inflammatory response and their survival is important for maintaining nasal homeostasis during inflammation. The fate of NECs depends on the balance between numerous pro-apoptotic and anti-apoptotic signaling. There is strong evidence that CRS is associated with the activated NEC apoptosis [20]. We believe that the pattern of Bcl-2 expression in NECs of patients with CRSsNP, namely more pronounced overexpression of anti-apoptotic and pro-survival Bcl-2 protein in atrophic regions observed in this study, aims at providing the survival of remaining NECs to maintain the integrity of damaged epithelial lining.

However, overexpression of Bcl-2 in multi-row, hyperplastic epithelium covering nasal polyps in patients with CRSwNP may be involved in sustaining proliferating NECs. Our hypothesis is consistent with other data that support the involvement of Bcl-2 in sustaining inflammation-induced hyperplasia of airway epithelial cells [21-23]. Nevertheless, the role of Bcl-2 overexpression in CRSwNP seems to be more important in damaged mucosal areas than in the proliferating ones, evidenced by a higher upregulation of Bcl-2 in NECs in regions with signs of damage to epithelial lining. Since the strongest Bcl-2 immunostaining in this study (both for CRSsNP and CRSwNP) was observed in the NECs where the damage to epithelia was the most significant and weak expression within NECs was found in undamaged areas, we believe that its upregulation is compensatory. It increases the viability of cells, which is crucial for preventing the extra involvement of nasal microbiota to the inflammatory process due to the loss of epithelial barrier.

Our findings are consistent with experimental data provided by Suo et al who demonstrated that nasal inflammation in rats is accompanied by overexpression of Bcl-2 mRNA and Bcl-2 protein in mucosal epithelia [24].

We speculate that Bcl-2 elevation in blood serum of patients with CRS found in this study is due to its release from damaged tissues. This can be partially confirmed by the trend, albeit statistically insignificant, towards a more pronounced increase in circulating Bcl-2 in CRSwNP compared with CRSsNP against the background of its higher expression in the nasal mucosa. However, this hypothesis has to be tested experimentally.

Taken together, our findings suggest that chronic nasal and paranasal inflammation may affect Bcl-2, mostly in CRSwNP, which can be considered an adaptive response to prevent cell loss.

Intense surface epithelial cell proliferation has been reported earlier in CRSwNP, evidenced by overexpression of proliferation markers such as Ki67 and proliferating cell nuclear antigen (PCNA) in the nasal mucosa. According to Mumbuc et al, such NEC proliferation in nasal polyps may contribute to the development of inverted papillomas [25]. We believe that Bcl-2 overexpression found in our study may also be involved in this process, since Bcl-2 is known to facilitate oncogenesis by inhibiting apoptosis [26]. However, more studies are required to study the role of Bcl-2 oveexpression in the development of tumors in the nasal and paranasal tissues.

This study has some limitations. Firstly, we did not analyze cell-specific markers in the lamina propria, which prevented us from identifying accurately the cells with overexpressed Bcl-2. Secondly, we did not have to opportunity to evaluate Bcl-2 mRNA expression. Nevertheless, immunohistochemical studies provided clear evidence of Bcl-2 overexpression in the nasal mucosa of patients with CRS. Thirdly, we had a limited number of patients. However, we managed to obtain statistically significant results. Fourthly, we could not determine circulating levels of pro-apoptotic BAX protein to assess the BAX/Bcl-2 ratio.

Conclusions

Both CRSwNP and CRSsNP are associated with overexpression of anti-apoptotic Bcl-2 in NECs and stroma against the background of elevated circulating levels of Bcl-2 protein. In CRSwNP upregulation of Bcl-2 in the nasal mucosa is more pronounced than in CRSsNP, while concentrations in blood serum don't differ. The study encourages the more detailed research on evaluating diagnostic and therapeutic implications of Bcl-2 in CRS.

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The factors affecting occurrence of urethral stricture after transurethral resection of the prostate

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Abstract

Objectives: Urethral stricture is one of the complex subjects of urology in terms of high recurrence rates, patient care, treatment difficulties and follow-up. We aimed to evaluate factors associated with the occurrence of urethral stricture after TUR-P (Transurethral resection of the prostate) surgery.

Material and methods: In our clinic, 301 patients who underwent TUR-P surgery for benign prostatic hyperplasia (BPH) were analyzed retrospectively. The patients who developed urethral stricture after TUR-P were named Group-1, did not develop were named Group-2. In addition, the patients were compared in terms of demographic and perioperative data.

Results: Urethral stricture was observed in 21 (6.97%) of the patients and not in 280 (93.03%) of them. There was no significant difference between the two groups in terms of age (p=0.913), resectoscope size (p=0.932), energy source type (p=0.932), energy source power (p=0.838), urethral catheter type (p=0.776), urethral catheter size (p=0.973), urethral catheter duration (p=0.797) and urethral catheter traction (p=0.887). Resection time was significantly higher in patients with urethral stricture (53.1±10.8 min vs. 42.2±9.7 min, p<0.001). The preoperative urinary tract infection (UTI) rate was significantly higher in patients with urethral stricture. (76.2% vs 40.0%, p=0.001). The optimum cut-off value for resection time associated with the risk of urethral stricture after TUR-P was 38.5 minutes, with an AUC of 0.812 (95% CI 0.738–0.885).

Conclusion: Prolonged resection time and even if treated, preoperative UTI increases the risk of urethral stricture after TUR-P surgery. However, if the resection time is not long, patients are more protected from developing urethral stricture.

Key words: TUR-P, urethral stricture, internal urethrotomy, prostate

Introduction

Guthrie first used transurethral resection (TUR) in urological surgeries in 1834. It has been developed and applied since the 1930s [1]. Currently, it is the gold standard method in staging and treatment of bladder tumors, treating benign prostatic hyperplasia (BPH), and relieving obstruction in advanced prostate cancer [2].

Urethral stricture is one of the complicated subjects of urology in terms of high recurrence rates, patient care, treatment difficulties and follow-up. Its etiology mainly includes iatrogenic causes and external traumas. The incidence of urethral stricture as a late complication of TUR is up to 9.8 % in studies [3]. Some studies have examined the factors that may cause urethral stricture after TUR [4]. However, the pathogenesis of stricture has not been clearly clarified.

In the current study, we aimed to investigate the urethral stricture seen after TUR-P surgeries performed in our clinic and the factors that may cause it.

Material and methods

Totally 301 patients who underwent TUR-P operation for BPH were analyzed. The patients were divided into two groups according to the occurrence of urethral stricture after TUR-P. The patients with urethral strictures were determined as Group 1, and those without urethral strictures were determined as Group 2. In addition, the patients were analyzed in terms of age, location of the stricture, the diameter of the resectoscope used during TUR-P, resection time, power and type of energy source used, urethral catheter duration, whether or not catheter traction was performed at the end of the operation and preoperative urinary tract infection (UTI).

TUR-P procedure was performed with 24 fr (Karl Storz) and 26 fr (Gyrus Acme) resectoscope sheaths. The conventional energy source was used in patients with 24 fr resectoscope sheath, and the plasmakinetic energy source was used in patients with 26 fr resectoscope sheath. Patients who were found to have urethral stricture during TUR-P were excluded from the study. Therefore, urethral dilatation was not performed in any patients before the procedure. Cathegell (12.5 g 2% lidocaine hydrochloride and 0.05 g chlorhexidine hydrochloride) was applied transurethrally before the resectoscope sheath was advanced from the penis. During the TUR-P procedure, 5% mannitol (resectisol) was used in patients who used a 24fr resectoscope as irrigation fluid, and saline (0.9% sodium chloride) was used in those who used a 26fr resectoscope sheath. After the procedure, an 18,20,22 fr latex or silicone Foley catheter was used. The TUR-P procedure was performed after the patients with preoperative UTI were treated with the appropriate antibiotic according to the urine culture.

Uroflowmetry was performed on patients who had complaints about postoperative urine flow rates. The patients with a curved pattern of stricture were evaluated with a 16 fr cystoscope under anesthesia. The patients with urethral stricture performed the Sachse model cold incision optic internal urethrotome developed by Karl Storz. 12 o'clock was chosen as the primary incision direction to avoid corpus cavernosum injury. All scar tissue visible to the proximal part of the stricture was excised entirely to the free connective tissue. Internal urethrotomy was performed under the guidance of a 3 fr ureteral catheter in cases with severe narrowness and multiple lumens.

Statistical analysis

Statistical analyzes were performed using SPSS Statistics software version 26.0 (IBM, Armonk, NY, USA). Categorical variables were stated as numbers and percentages. Continuous variables were expressed as means, standard deviations, medians and interquartile ranges according to the normality of the distribution. Comparison of continuous variables was done with Student's T-test/Mann-Whitney U tests. Categorical variables were compared with Chi-square and Fisher's exact tests. Spearman correlation analysis was used to define the association between urethral stricture and perioperative parameters. Receiver operator characteristics (ROC) curve analysis was applied to define optimum thresholds via area under the curve (AUC). The optimum threshold value for the resection time for urethral stricture was determined by the Youden Index on the ROC curves. P-value <0.05 was defined as statistical significance.

Results

The urethral stricture was observed in 21 (6.97%) patients who underwent TUR-P, and it was not observed in 280 (93.03%) of them. The mean age were 70.9±7.8 years in Group 1 and 70.6±7.4 years in Group 2 (p=0.913). There was no significant difference between the two groups in terms of resectoscope size (p=0.932), energy source type (p=0.932), energy source power (p=0.838), urethral catheter type (p=0.776), urethral catheter size (p=0.973), urethral catheter duration (p=0.797) and urethral catheter traction (p=0.887) (Table 1).

Table 1 Demographic and per-operative data of patients according to urethral stricture status

	Group 1 (n=21)	Group 2 (n=280)	P value
Age (years), mean±SD	70.9±7.8	70.6±7.4	0.913
Resection time (minutes), mean±SD	53.1±10.8	42.2±9.7	**<0.001**
Resectoscope size, n (%) 24F 26F	5 (23.8) 16 (76.2)	69 (24.6) 211 (75.4)	0.932
Energy source type, n (%) Conventional Plasmakinetics	5 (23.8) 16 (76.2)	69 (24.6) 211 (75.4)	0.932
Energy source power, n (%) <150 watt >150 watt	8 (38.1) 13 (61.9)	113 (40.4) 167 (59.6)	0.838
Urethral catheter type, n (%) Latex Silicon	16 (76.2) 5 (23.8)	205 (73.2) 75 (26.8)	0.776
Urethral catheter size, n (%) 18F 20F 22F	5 (23.8) 13 (61.9) 3 (14.3)	64 (22.9) 180 (64.3) 36 (12.9)	0.973
Urethral catheter duration (days), mean±SD	4.6±1.6	4.4±1.6	0.797
Urethral catheter traction, n (%)	15 (71.4)	204 (72.9)	0.887
Preoperative UTI, n (%)	16 (76.2)	112 (40.0)	**0.001**

Figure 1 - Number of urethral strictures according to preoperative UTI

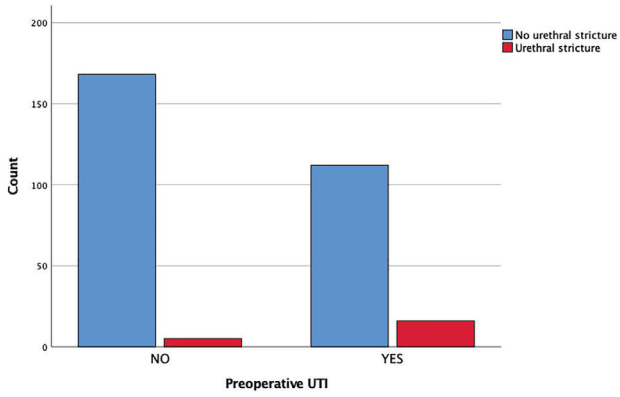

Table 2 Spearman correlation analysis between the urethral stricture and per-operative parameters

Characteristics	Urethral stricture	
	rho value	P value
Age	0.001	0.987
Resection time	0.297	**<0.001**
Resectoscope size	0.005	0.932
Energy source type	0.005	0.932
Energy source power	0.012	0.839
Urethral catheter type	-0.017	0.767
Urethral catheter size	0.001	0.981
Urethral catheter time	0.045	0.434
Urethral catheter traction	-0.008	0.888
Preoperative UTI	0.186	**0.001**

The resection time were significantly higher in patients with urethral stricture (53.1±10.8 min vs. 42.2±9.7 min, p<0.001). Similarly, the preoperative UTI rate was significantly higher in patients with urethral stricture. (76.2% vs 40.0%, p=0.001) (Figure 1). In Spearman correlation analysis, both resection time (rho: 0.297, p<0.001) and preoperative UTI (rho: 0.186, p=0.001) had a positive and significant relationship with the urethral stricture occurrence (Table 2). ROC curve analysis was performed to determine the optimum cut-off value for resection time associated with the risk of urethral stricture after TUR-P. It was assigned 38.5 minutes, with an AUC of 0.812 (95% CI 0.738–0.885). The highest sensitivity and specificity were 0.857 and 0.626, p<0.001 (Figure 2).

Figure 2 - Receiver operating characteristic (ROC) curve analysis of the resection time for urethral stricture

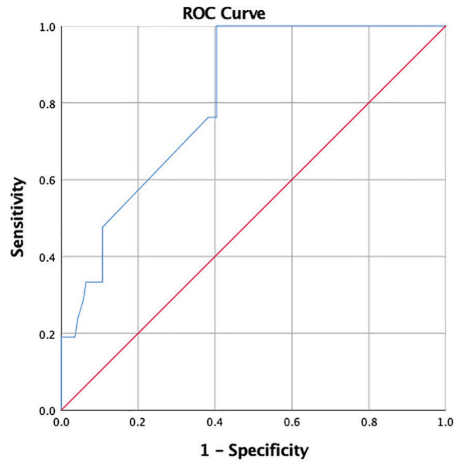

Table 3 Number of patients according to urethral stricture localization

Urethral stricture localisation	N (%)
Bulbous	12 (57.1)
Membranous	6 (28.5)
Penile	3 (14.2)

The urethral stricture was observed in the bulbar, membranous and penile urethra in 12 (57.1%), 6 (28.5%) and 3 (14.2%) patients, respectively (Table 3).

Discussion

The incidence of urethral stricture after TUR-P is similar to previous years, despite improvements in surgical techniques, technology, and lubricants. For example, Tan GH et al. found a urethral stricture rate of 3.5% in long-term follow-up of 373 patients who underwent TUR-P surgery [5]. Similarly, Afandiyev F. et al. found the rate of urethral stricures after TURP as 10.5% in their study of 124 patients who were followed up [6]. In addition to these two previous studies, Grechenkov A. et al. reported the rate of urethral stricture as 8.6% in their current study with 402 patients who underwent bipolar TUR-P [7]. In our research, the urethral stricture rate after TUR-P was 6.9 %, consistent with the literature.

Previously, the most common cause of urethral stricture was urethritis caused by sexually transmitted diseases [8]. In contrast, nowadays, reasons such as TUR, cystoscopy, urethral catheterization, prostatectomy, brachytherapy and hypospadias surgery come to the fore [9].

While complications such as bleeding, bladder perforation, prostate capsule perforation, TUR syndrome and intraoperative priapism can be seen in the early period after TUR-P, complications such as bladder neck contracture and urethral stricture may occur in the long term [10,11]. Urethral stricture can be defined as scar formation in the urethral mucosa and surrounding tissues. Any procedure that may cause trauma to the urethra can result in urethral stricture. It is thought that the friction of the penoscrotal angle in the bulbous urethra with the resectoscope sheath during the TUR-P procedure, especially at the first entry and during the subsequent operation, causes mucosal damage, urine leakage into the submucosal area, and eventually scar tissue formation and thus urethral stricture formation. Applying sufficient lubricant to the urethra and resectoscope sheath before TUR-P will reduce friction between the urethra and the resectoscope sheath. In the studies conducted by V.Mauermayer, it has been reported that the lubricant used during the TUR procedure makes the electrical leakage from the resectoscope more conductive. If the electrical permeability of the lubricant used is less than the permeability of the urethral tissue around the resectoscope shaft, electricity enters the generator correctly from the neutral plate. Otherwise, an electric current passes through the urethra to the body at the point of electric leakage and causes stricture [12]. When the resectoscope sheath is advanced in the penis, especially during the passage through the bulbous urethra, under direct observation and sufficient lubricant will reduce the development of urethral stricture.

Studies show that the risk of developing urethral stricture increases as the resection time increases during the TUR-P procedure [13,14]. Our research observed that the risk of urethral stricture increased as the resection time increased. Since prolonging the resection time will increase the risk of urethral trauma, not keeping the resection long, especially in patients with large prostate volume, completing the TUR procedure in more than one session, if possible, will protect the patients from the development of urethral stricture.

In the study performed by Balbay et al. on 103 patients in 1992, it was reported that the rate of urethral stricture development after TUR increased as the patient's age increased [15]. However, in our study, no relationship was found between the age of the patient and the development of urethral stricture.

Although it was reported that there is a significant relationship between the diameter of the resectoscope and the development of stricture according to the studies performed by Tefekli et al. [16], no association was found between the diameter of the resectoscope and the development of urethral stricture in our research.

In some studies, it has been reported that the diameter of the catheter used after the operation, the type of catheter and the length of the catheter stay affect the development of stricture [17,18]. However, in our study, no relationship was found between these factors and stricture development.

According to the studies of Zaid UB et al., it was reported that the presence of UTI before TUR increases the risk of developing urethral stricture [19]. Similar results were obtained in a recent study conducted in 2017 [20]. In our study, 128 patients had preoperative UTIs, and 16 of them (12.5%) developed urethral stricture after TUR-P. In our study, the presence of preoperative UTI caused stricture development at a high rate, although it was treated with appropriate antibiotics according to the urine culture. Due to the development of multiple resistance to drugs in diabetic patients, more attention should be paid to UTI [21].

It has been reported that high energy use during TUR increases the risk of urethral stricture [22]. However, in our study, no relationship was found between the power of the energy source and the development of urethral stricture. Likewise, in our study, no association was found between the application of catheter traction at the end of the operation and the development of stricture.

Treatment of urethral stricture varies according to the location, length and shape. The most commonly used technique in surgical treatment today is the cold incision endoscopic internal urethrotomy technique applied by Hans Sachse [23]. In addition, urethral dilatation and Holmium-YAG laser applications are also included [24-26]. Our study treated patients who developed urethral stricture with endoscopic internal urethrotomy.

Study limitations

Our study was limited because it was performed in a single center and retrospectively. Therefore, multicentre, more comprehensive and prospective studies are needed to verify our findings.

In conclusion, despite technological advances, urethral strictures are still high after TUR-P. Therefore, even if preoperative UTI is treated, it should be considered that it increases the risk of stricture formation. However, in the TUR-P procedure, if the resectoscope is made with sufficient lubricant and under direct observation, care is taken to avoid electrical leakage from the resectoscope, and the resection time is not kept too long, patients will be more protected from the risk of developing urethral stricture.

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Attitudes of non-physician health workers working in the Emergency Department towards euthanasia, death, and the terminally patient

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Abstract

Aim: The use of the right to die in the center of the individual's own decision is called euthanasia. This decision, was evaluated from legal, religious, and medical perspectives. In different countries applied euthanasia, which can be performed actively or passively. In our study, we planned to investigate the perspectives of healthcare professionals working in the emergency department about euthanasia and their thoughts on diseases that can be applied to euthanasia.

Material and methods: A survey was conducted from June to October 2022 on non-physician health workers working in the Emergency department. A questionnaire including demographic data, professional knowledge, and Attitude Scale towards Euthanasia, Death, and the Terminally Patient was administered to the healthcare professionals who agreed to participate in the study. The obtained data were analyzed.

Results: In the study, the feedback of 60 participants, 37 of whom were women, was evaluated. The mean age of the entrants was found to be 39.07±10.11 years. 60% of the participants had received cardiopulmonary resuscitation training in the past year. 70% of the participants stated that they could be euthanized for coma, 38.33% for severe disability, and 36.67% for severe and incurable neurological diseases.

Conclusion: In the process of euthanasia, which does not have a legal infrastructure in our country, different perspectives are seen from different departments of health services.

Key words: emergency department, euthanasia, non-health workers

Introduction

Euthanasia: It is an issue that concerns many different sciences, especially religion, law, and medicine, and has been discussed for many years. It is defined as the right to die by the Turkish Language Association [1]. In euthanasia, distinctions have been made according to criteria such as the method of application, the decision-maker, the will of the person, and time, and there may be differences according to countries in the legal context under different conditions and practices [2,3]. Active euthanasia is performed directly by the physician using a medical method, while passive euthanasia is expressed as not providing the necessary support to the patient to prolong the patient's life span.[4].

Euthanasia is practiced in many countries in the world, and active euthanasia is prohibited in the USA, and it is free if passive. In European countries such as the Netherlands and Belgium, euthanasia is legal but not legal in the UK. In our country, both active and passive euthanasia is banned [5]. The regulation on euthanasia in the Turkish legal system is also "Euthanasia is prohibited. The right to life cannot be waived for medical reasons or by any means whatsoever. No one's life can be ended, even if he or someone else demands it." is in the form. Even though it is not legally accepted, euthanasia has been the subject of discussion in our country for many years in the legal and criminal sense [6].

The right to life is the most basic right of the individual and is accepted all over the world, countries are obliged to protect it and take the necessary measures. If euthanasia is accepted as the right to die, the problem of violation of the right to life arises among the rights that cannot be disposed [7,8]. The most important aim of medical science is to save human health and find a cure for diseases, and in this direction, medicine is constantly renewing itself [9]. It will be important to include the opinions of non-physician health personnel, who are

among the most basic employees in health care, on euthanasia issues, in terms of discussing both ethical and legal problems in this regard. In this study, it is critical to determine the opinions of non-physician health professionals working in health care on euthanasia in terms of contributing to the literature. Therefore, in this study, we aimed to determine the ideas of non-physician healthcare professionals on euthanasia.

Material and methods

This study was carried out with the approval of the ethics committee of Health Science University Antalya Training and Research Hospital (Date: 16.06.2022, decision no: 12/10) on non-physician assistant health personnel (nurse, midwife, paramedic, health officer) working in the emergency department of a 3rd level university hospital. The study was conducted following the Principles of the Declaration of Helsinki. Participants who were assigned to the emergency department and performed the specified tasks and agreed to participate in the study were included in the study. Participants with incomplete information or who did not agree to participate in the study were excluded from the study. A questionnaire consisting of demographic data such as gender, age, tenure, education, titles, information on euthanasia, and Attitudes towards Euthanasia, Death, and the Terminally Patient (EDTP) was distributed to the participants [10]. The questionnaires that were withdrawn from the participants and that met the inclusion criteria were analyzed by hiding the identity of the participants. After the exclusion criteria, 60 non-physician health workers who accepted to participate in our study were included in the study.

EDTP scale application

The EDTP scale was developed by Şenol et al. in 1996 and the Cronbach Alpha value was found to be 0.84 [11]. Our article Cronbach Alpha value was 0,783. It consists of 31 questions answered with a 4-point Likert scale. Questions 3,6,7,8, 10, 12, 13, 15, 16, 17, 20, 23, 24, 27 in the scale are reverse scored. The 5-factor analysis specified in the study published by Şenol et al. in 1996 was carried out [11]. The distribution of the factors is given in Table 1.

Table 1 Attitude scale factors and question distributions regarding euthanasia, death, and the terminally patient.

Factor	Questions	Cronbach Alfa	Answer Means± SD
Total	-	0,783	78.5±11.4
1: Attitudes about euthanasia	14, 19, 26, 6, 29, 12, 22, 16, 1, 18, 2, 9, 5, 21	0,899	32.53±10.31
2: Feelings about the terminally ill and her family	7, 24, 23, 8, 28, 3	0,577	18.83±2.99
3: Thoughts on death attributed to patients	30, 21, 25, 15, 4	0,086	11.98±1.94
4: Avoidant attitudes towards death	17, 15, 10, 13, 27,8	0,431	17.52±2.75
5: Non-avoidant attitudes towards death	13, 20, 11, 23, 1, 28	0,251	15.48±2.67

Statistical analysis

The obtained data were analyzed in an appropriate Microsoft SPSS 23.0 software package statistical program. The findings were analyzed at a 95% confidence interval and a 5% significance level. In the evaluation of the data; number and percentage in categorical data as descriptive statistical methods; mean, standard deviation, median, and minimum-maximum were used in numerical data. In the statistical analysis, firstly, whether the groups were suitable for normal distribution was examined Student's T-test and Mann-Whitney U tests were used in the evaluation of numerical data, and the chi-square test was used in the evaluation of categorical data. Data with a p-value of 0.05 and below were considered significant.

Results

A total of 60 people, 37 (61.67%) of whom were women, participated in our study. The mean age of the participants was found to be 39.07±10.11 years. 21% of the participants stated that they had worked in an intensive care unit before, and 40 (66.67%) participants said that they had previously received training or courses for working in the emergency room. The demographic data of the participants, their professional experiences, and their perspectives on euthanasia are given in Table 2.The demographic data of the participants and their EDTP scores and factors were compared. In the comparison, a significant difference was found between age groups for factor 5, in which non-avoidant attitudes towards death were evaluated (p=0.0208).

The duration of the advanced life support training attended by the participants in the last 1 year and Factor 4, which evaluates avoidance attitudes towards death, were compared, and the EDTP score of those who received training in the last 1 year was found to be significantly higher (p=0.0394). In Factor

Table 2 Demographic data of healthcare workers.

Demographic Data	Mean	SD
Age	39.07	10.11
Work time (Year)	16.98	10.39
Sex n(%)	N	%
Female	37	61.67
Male	23	38.33
Profession n(%)	n	%
Nurse	41	68.33
Midwife	6	10.00
Emergency Medical Technician (EMT)	6	10.00
Medical Officer	7	11.67
Education n(%)	n	%
High-Scholl	4	6.67
Associate Degree	17	28.33
Bachelor's degree	35	58.33
Master of Science, Master's Degree	4	6.67
Questions	n	%
People who previously worked in intensive care	21	35.00
Persons receiving training/courses for the Emergency Service	40	66.67
Last "Advanced Life Support" training time n(%)		
0-1 year	36	60.00
1-2 years	5	8.33
2-3 years	2	3.33
3+ years	17	28.33
Those who know euthanasia n(%)	49	81.67
Is euthanasia legal in Turkey? n(%)	0	0.00
Who wants euthanasia around or near n(%)	6	10.00
Conditions (Euthanasia is applicable) n(%)	n	%
Severe/incurable neurological diseases	22	36.67
Incurable infections	7	11.67
Disabilities	23	38.33
Severe traumas	8	13.33
Coma	42	70.00
Cancer	14	23.33
Psychotic Illnesses	6	10.00
Neurotic Diseases	8	13.33

Table 3 Attitude scale towards euthanasia, death and the terminally illness score and comparison of the answers according to the factors.

Specification	n	Total	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Age							
20-29	15	72.8±9.67	27.6±8.48	18.2±2.93	12.2±1.52	17.53±2.67	14.27±2.66
30-39	12	77.33±10.34	32.58±9.85	17.92±3.99	11.5±1.45	17.33±3.31	14.42±2.35
40-49	26	80.58±11.75	34.35±10.54	18.96±2.55	11.85±2.03	17.62±2.65	16.31±2.59
50+	7	85±12.06	36.29±12.02	21.29±1.25	12.86±3.02	17.43±2.88	16.86±2.12
F value		2,499	1,791	2,34	0,8162	0,02992	**3,517**
P value		0,0689	0,1593	0,0831	0,4903	0,993	**0,0208**
Works experience (year)							
0-9	16	74±11,01	29,69±10,16	17,69±3,77	11,5±1,27	17,44±3,22	14,31±2,77
10-19	21	78,71±1,92	32,05±8,59	19,29±2,43	12,14±1,88	17,52±1,99	15,52±2,21
20-29	16	80,19±13,5	34,81±11,31	18,38±2,78	11,81±2,01	17,38±3,24	16,19±3,04
30+	7	84,29±13,06	35,29±13,25	21,14±1,46	13±3,06	18±2,94	16,43±2,37
F value		1,587	0,8421	2,676	1,059	0,08633	1,754
P value		0,2028	0,4766	0,0558	0,3737	0,9672	0,1665
Profession Group							
Nurse	41	78,41±11,55	32,93±9,87	18,59±3,08	12,12±1,95	17,24±2,72	15,46±2,97
Midwife	6	83,17±14,68	34,67±14,04	20,67±1,21	12,17±2,48	19,67±3,14	16±1,9
Emergency medical technician	6	72,17±10,34	27,33±9,95	17±2,90	12±1,27	17,67±3,27	14,17±1,47
Medical Officer	7	80,43±7,93	32,86±10,88	20,29±2,56	11±2	17,14±1,58	16,29±1,98
F value		1,014	0,6043	2,295	0,673	1,435	0,7627
P value		0,3936	0,615	0,0877	0,5722	0,2424	0,5197
Intensive care work history							
No	39	78,33	32,15	19,15	11,79	17,38	15,49
Yes	21	78,81	33,24	18,24	12,33	17,76	15,48
P value		0,8793	0,7011	0,2608	0,3102	0,6167	0,988
Have you taken a nursing course in the emergency department?							
No	20	80,3	33,65	18,6	11,75	18,05	16,2
Yes	40	77,6	31,98	18,95	12,1	17,25	15,13
P value		0,3934	0,5575	0,6725	0,5156	0,2924	0,1431
When was the last time you received advanced life support training?							
0-1 year	36	77,86	31,36	18,94	11,86	18,11	15,28
1+ years	24	79,46	34,29	18,67	12,17	16,63	15,79
P value		0,6005	0,2845	0,7274	0,5554	0,0394	0,4701
Do you know about euthanasia?							
No	11	84,36	38	19,36	12,27	17,73	15,55
Yes	49	77,18	31,31	18,71	11,92	17,47	15,47
P value		0,0593	0,0508	0,5193	0,5892	0,7815	0,9328
Have you or someone close to you requested euthanasia?							
No	54	79,37	33,31	18,87	12,22	17,44	15,63
Yes	6	70,67	25,5	18,5	9,833	18,17	14,17
P value		0,0768	0,078	0,776	0,0034	0,5466	0,2058

3, in which thoughts about death attributed to patients were examined, it was found that people who did not have relatives or family members who wanted euthanasia had a significantly higher EDTP score than those who had EDTP (p=0.0034). The issuance of the questions according to the factors is given in Table 3.

In the comparison of the participants' perspectives on diseases and the implementation of euthanasia, the EDTP scores of those who did not want euthanasia in all disease categories were found to be significantly higher than those of those who wanted it. The allocation of scores is given in Table 4. In the analysis of multiple variations between the EDTP score and the responses to diseases, statistically significant results were found for patients with severe and incurable neurological diseases, incurable infections, and cancer. Multivariate analysis of variance (MANOVA) of disease groups to attitude scale toward is given in Table 5. A positive correlation was found for both variables in the correlation analysis between the participants' ages and working time and their EDTP scores (age: R2= 0.09162 p=0.0187, working year: R2=0.07993 p=0.0286).

Table 4 Comparison of attitude scale scores and factor scores regarding euthanasia, death, and the terminally patient according to diseases.

Specification	n	Total	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Severe neurological diseases, incurable neurological diseases							
No	38	83,34	36,79	19,42	12,24	17,32	15,87
Yes	22	70,14	25,18	17,82	11,55	17,86	14,82
P value		<0,0001	<0,0001	0,0442	0,1867	0,4622	0,1436
Incurable infections							
No	53	79,98	33,77	18,98	11,94	17,43	15,79
Yes	7	67,29	17,71	17,71	12,29	18,14	13,14
P value		0,0048	0,0001	0,2955	0,6653	0,5265	0,0124
Severe disability							
No	37	81,22	35,22	19,11	12,05	17,03	15,76
Yes	23	74,13	28,22	18,39	11,87	18,3	15,04
P value		0,0183	0,0094	0,3706	0,7241	0,0804	0,3187
Severe and advanced trauma							
No	52	79,96	33,88	19,02	11,94	17,25	15,69
Yes	8	69	23,75	17,63	12,25	19,25	14,13
P value		0,0104	0,0085	0,222	0,6805	0,055	0,1233
Coma							
No	18	86,06	38,89	19,44	13	17,67	16,78
Yes	42	75,26	29,81	18,57	11,55	17,45	14,93
P value		0,0005	0,0013	0,3035	0,0069	0,7849	0,0127
Cancer patients							
No	46	80,52	34,67	19,02	11,85	17,07	15,87
Yes	14	71,86	25,5	18,21	12,43	19	14,21
P value		0,0118	0,0028	0,3803	0,332	0,0199	0,0413
Those with psychotic illness							
No	54	79,56	33,3	19,15	11,96	17,39	15,69
Yes	6	69	25,67	16	12,17	18,67	13,67
P value		0,0308	0,0855	0,013	0,81	0,2845	0,0789
Those with neurotic disease							
No	52	79,65	33,69	19,02	12	17,29	15,71
Yes	8	71	25	17,63	11,88	19	14
P value		0,0454	0,0251	0,222	0,8672	0,102	0,0917

Table 5 MANOVA of disease groups according to attitude scale towards euthanasia, death, and the terminally patient.

	EDTP Score	0	1	2	3	4	5	6	7	8	P Value	Estimate	Standard error	95% confidence interval
0		1									**<0,0001**	89,08	2,226	84,61 to 93,55
1	B: Severe/ incurable neurological diseases	-0,145	1								**0,0011**	-10,51	3,036	-16,60 to -4,414
2	C: Incurable infections	-0,07506	-0,3413	1							0,5568	-3,157	5,336	-13,87 to 7,556
3	D: Disability	-0,05741	-0,3351	0,2269	1						0,3636	3,003	3,277	-3,575 to 9,581
4	E: Severe Trauma	0,09022	-0,05138	-0,3505	-0,2067	1					0,9757	0,1666	5,441	-10,76 to 11,09
5	F: Coma	-0,7073	-0,1021	0,1299	-0,1829	-0,09279	1				**0,0014**	-9,134	2,695	-14,54 to -3,725
6	G: Cancer	-0,1082	0,05991	-0,2249	-0,4075	-0,03366	0,06119	1			0,1801	-4,895	3,602	-12,13 to 2,336
7	H: Psychotic illnesses	-0,0458	0,03899	-0,1153	0,05224	-0,5209	0,04187	0,1074	1		0,7003	-2,635	6,807	-16,30 to 11,03
8	I: Neurotic diseases	0,0723	-0,00571	-0,1203	-0,1356	0,2388	-0,08874	-0,2558	-0,5841	1	0,7074	1,974	5,231	-8,527 to 12,48

Discussion

The right to life, one of the most fundamental rights of individuals, must be guaranteed for other rights to be valid. For this reason, countries with modern democracy must guarantee this with the constitution. For many years, euthanasia has brought science and ethics against each other, and it often causes disagreement among scientists [12,13]. Studies have illustrated that healthcare professionals assume they have the competence to manage this demand in countries where euthanasia is legal. In this sense, there have been requests for inclusion in euthanasia as a part of professional qualification in countries where this type of euthanasia is legal [14].

A study on neurological conditions evaluated cases of euthanasia and assisted suicide in the Netherlands, Germany, and Switzerland. When the cases between 2001 and 2013 are analyzed, it is seen that there is an upsurge in euthanasia issues. Euthanasia applications are most frequently performed in cancer patients and then in patients with neurological system involvements [15-17]. Makish et al. focused on the outcome of psychiatric patients with euthanasia or assisted death. The legal infrastructure for euthanasia in the Netherlands was regulated in 2002 [17]. Only 1% of euthanasia performed in the Netherlands was reported as psychiatric [18]. It requested euthanasia between 1100 and 1150 between 2015 and 2016 in Germany, and 60-70 of them were of psychiatric origin [19]. The number of cases reported in Belgium was 2655 people in 2019, 49 of them had psychiatric indications. While the number of cases reported in the last five years was 61 in 2014, it was stated as 49 in 2019 [17-19]. In our study, statistically significant results were found for patients with severe and incurable neurological diseases, incurable infections, and cancer, in the analysis of multiple variations between the PTS score and the responses to diseases.

Another issue for euthanasia and assisted death is a disability. The problem of euthanasia requests and the realization of patients who have a high level of loss in anatomical and mental capacity and whose life is difficult has been a subject of discussion. Detailed information was published for 416 of 259301 patients who applied for euthanasia during the five years between 2012 and 2016 [20]. In the subject of the conditions determined by the committee, euthanasia conclusions are made on cognitive disorders and unavoidable disability situations such as progressive dementia. Applications in this field have been regarded as diseases that cause psychiatric and neurological disabilities since childhood. In addition, events that completely disrupt mental and social health are at the forefront of chronic processes such as life-disrupting chronic obstructive pulmonary disease and heart failure, childhood rape, and subsequent suicide attempts and mental problems [21]. In our study, if the evaluation is made for euthanasia and assisted death, severe and incurable neurological diseases, incurable infections, and cancer patients were determined.

Conclusion

Euthanasia, which is not yet legally valid in our country, but is applied in selected diseases and patients in some countries on earth, is an ethical sensation for the right to life of the individual. Regardless, emergency services, where emergencies are resolved in the follow-up and treatment of the patient, do not have sufficient observance and evaluation conditions for the making and execution of euthanasia decisions. With this and identical studies, the attitudes and expectations of both the healthcare professionals working in the emergency department and the patients on this issue can be analyzed.

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Preparation of the surgical place for laparoscopic procedure of the prostate gland tumors

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Abstract

Aim: The purpose of this study to present the upgrading method of laparoscopic procedure of prostate cancer.

Material and methods: This work was made as part of the PhD dissertation. This technique could be recommended for treatment of the prostate gland tumors. The Patent of the Republic of Kazakhstan №35437 was received on 31.12.2021

Results: The laparoscopic procedure for both methods are the same, there is some upgrading of traditional method by proposing improvements of trocars insertion. During traditional method four working trocars are installed extraperitoneal under the control of optics. We recommend four working trocars are installed under the control of the index finger, palpating the lower epigastric vessels from the inside; it is avoid to damage of the peritoneum, injection of gas into the abdominal space, damage of the vessels of the pelvis.

Conclusion: The method allows avoiding bleeding, pneumoperitoneum, and decreased saturation; and in the early postoperative period, peritonitis is excluded and the late postoperative period, adhesive processes do not occur.

Key words: laparoscopic procedure, extraperitoneal radical prostatectomy, prostate cancer, Kazakhstan

Introduction

The pioneers of endoscopic extraperitoneal radical prostatectomy were Schuessler and colleagues who performed the first 9 cases of this procedure [1]. In the beginning endoscopic extraperitoneal radical prostatectomy caused a lot of doubts and questions due to the long duration and the scale of difficulty. Since then it has undergone many modifications. Guillonneau and Vallancien described a technique (LPR Montsouris technique) which allowed performing surgery in less than three hours [2]. New solutions were introduced for avoiding complications associated with the transperitoneal route [3,4]. Preperitoneal access was proposed by Raboy and colleagues [5]. This way was used and justificated a series of 42 cases by Bollens and co-workers in 2001[6].

Comparation between laparoscopic procedures and open procedures give more advantages for laparoscopic procedures due to shorter postoperative hospital stay, faster return to physical activity and through advanced optical systems, better vision of the operative field [7]. For patients with localized prostate cancer laparoscopic radical prostatectomy is the best way for treatment [8]. LPR has become a first line treatment for patients with localized prostate cancer, in many centers around the world. Diagnostic surgeries and reconstructive operations can be made by urological laparoscopy. Laparoscopic technic has many benefits such as lower consistency

risk and faster recovery time after surgery. But this operation is fraught with some complications during entry to abdomen including visceral injury, urological tract injury, hemorrhage, herniation and infection [9]. Basically, complications occur when the laparoscope is entry to abdominal wall. These complications could follow to mortality. However, a very important advantage of laparoscopy is saving time. Reducing of entry time for performing of laparoscopy could decreased overall surgery time and complication it is also reduce of anesthesia and general surgery.

For laparoscopic surgery different approaches have been created nowadays [10,11]. A few international investigations have proposed principal of safe laparoscopic entry [12-16]. Open laparoscopy named as Hasson and close, direct entry laparoscopy is used in general surgery [17]. Benefits of open entry are due to low probability of vascular injury [18]. However, this technique involves some complications. To avoid these complications, optical-controlled trocars are offered, reducing the risk of injury with intra-abdominal construction, allowing the surgeon to observe the placement of abdominal structures [18]. The Visiport optical trocar is a disposable and expendable visual entry tool which includes a cannula and hollow trocar. It is enter after injection of CO2. This method is palmed via surgeon's hand and supported perpendicular to distend patient's CO2 to abdomen [19]. When correct anatomical statute of trocar tip is verified by monitor, downward axial pressure is used and the trigger is activated. Downward pressure causes trocar tip situation is checked again. These series are repeated till the peritoneal cavity is reach. This is not fired till the accurate anatomical status of trocar tip is known. However, none of the laparoscopic entry techniques have obvious advantage over others. All of these methods are connected with numerous complications [20]. We propose the way of upgrading of laparoscopic entry techniques.

The aim of this article to present the upgrading method of laparoscopic procedure of prostate cancer.

Material and methods

To meet the general trend in the World Urology, interests and expectations of patients associated with laparoscopic procedures, as well as, the growing importance of laparoscopy in Kazakhstan, we also started to implement laparoscopic procedures in the East Kazakhstan region multi-profile "Center of Oncology and surgery". We made many laparoscopic procedures in patients with prostate cancer and during surgery tried improve this method. We propose the way of upgrading of procedure as a part of the PhD dissertation. Before treatment all patients provided written Informed Consent after providing detailed information about our method and traditional laparoscopic procedures, and all outcomes were explained. Only after the patient's voluntary consent, the operation was performed by using our method. The study received approval from the Semey Medical University Ethics Committee (Protocol № 2, October 18, 2019). The Patent of Republic of Kazakhstan №35437 was received in 31.12.2021.

This technique could be recommended for treatment of the prostate gland tumors. There is a known method of laparoscopic extraperitoneal radical prostatectomy, when a cavity is formed between the muscles of the anterior abdominal wall and the peritoneum before surgery. During the extraperitoneal technique, a 2 cm incision is made along the midline 1 cm below the navel. After opening the anterior leaflet of aponeurosis and retraction of the rectus abdominis muscle, a finger dissection is performed to access the space of Retzius. Then a dissector balloon is inserted in the direction of the bosom, into which up to 800 ml of gas is insufflated under visual control. After creating the working space, the balloon-dissector is removed, an optical trocar is placed. Four working trocars are installed extraperitoneal under the control of optics. No.1, 2 along the pararectal line in the area of the iliac spines on the right is 5mm and on the left is 10mm. Then, a standard prostatectomy is performed according to a well-known technique. [1,5,21].

But this method has some disadvantages. Trocars in 40-60% damage the peritoneum and gas is pumped into the abdominal cavity which follows to unfavorable outcomes such as pressuring on the diaphragm, bladder is pushed into the surgical field, and there is high possibility to damage of the lower abdominal and iliac vessels [20].

Case presentation

Patient present in hospital 27.11.2019, male, 66 years old, non-smoker

Patient medical history. No hepatitis. No surgery procedures. No blood transfusions

The patient's and his family's medical history. Mother – n.a. Father – n.a.

Symptoms. He had complaints on difficulty urination, weakness, painful urination, and feeling of incomplete emptying of the bladder.

Case history. These symptoms have disturbed him during one year. He has been observed by urologist. It is noted that condition has deteriorated in dynamic.

Physical examination:

Weight – 98 kg

Height – 164sm

BMI – 36.4 kg/m2

Physical examination – unremarkable

X-Ray examination – unremarkable

ECG: Sinus rhythm. HR is 67 per minute. There is normal position of EAH.

Cardiologist: Arterial hypertension II degree, Risk 4. HF1

Histological analysis: Acinar adenocarcinoma of the prostate gland with perineural and vascular invasion, tumor growth is observed on the tip; there are no regional metastases in the lymph nodes. The tumor growth in the seminal vesicles is revealed

Date of surgery 29.11.2019. Laparoscopic prostatectomy with urethro-vesical anastomosis. Pelvic lymph dissection. Surgery procedure was made by our method.

Postoperative therapy.

Nacl 0.9%-500.0 + Euphyllin 10.0 No.3, Nacl 0.9%-200.0 +Tugina 500mNo.2, Furosemide 20mg No.4, Ketorol 2,0x3time a dayNo.3, Proserin 0,5x3time a dayNo.3, Ceftazidim 1.0 No.8, Fortrans 2 pack. He was in the ICU for 1 day after surgery. Postoperative period without complications, the drainage tube of the paravesical area was removed on 4 days. The catheter was removed on day 7.

Final diagnosis. C-61: Prostate cancer. T2нхмо. Arterial hypertension 2 degree. Risk 4. CHF1. Obesity second degree

Patient had satisfactory condition during checking out. There is vesicular breathing in the lungs. Heart tones are muted, rhythmic BP is 130\80 mmHg. The tongue is moist. The abdomen is regular in shape, symmetrical, participates in the act of breathing. On palpation, the abdomen is soft. There are no peritoneal signs. Liver is along the edge of the costal ribs. The spleen is not palpable. The symptom of the pounding is negative. He has not have constipation. The diuresis is independent. The Karnovsky index is 80 points.

Treatment outcome: Recovery. Patient checked out on 08.12.2019. 11 (11 days)

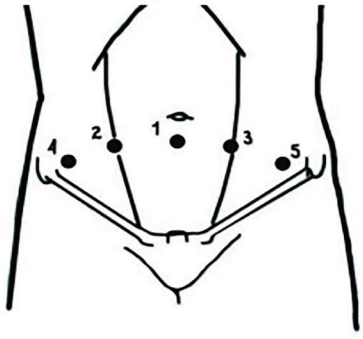
Table 1 Laboratory data, and diagnostic procedures

	Normal	Unit	First	Last
BLOOD ANALYSES				
Erythrocytes	4.30-5.80	10^12/µl	5.79	4.64
Hemoglobin	140-180	g/l	145	126
Leucocytes	4.000-9.000	10^9 µl	7.1	6.3
ESR	2-15	mm/h	-	41
Platelets	140-400	10^9 µl	249	220
Bilirubin	3.4-21.0	mkmol/L	23.8	14.63
ASAT	10-50	U/l	-	26
ALAT	10-50	U/l	-	19
Total protein	64-83	g/L	77.5	68.2
Glucose	3.3-5.6	mmol/L	-	6.7
Urea	3.2-8.3	mmol/l	-	10.2
Creatinine	62-106	mkmol/L	109	101.3
URINE TEST				
Specific gravity	1010-1023		1010	1024
Leucocytes	0-3	in sight	1-2	Completely
Protein	-	g/L	-	1.65
Epithelium	0-3	in sight	1-2	3-4
COAGULOGRAM				
INR	0.85-1.15	Unit	1.03	-
ADDITIONAL TEST				
HIV	Negative		Negative	
Microreaction	Negative		Negative	
Hepatitis B, C	Negative		Negative	

Discussion

Our invention solves the problem of damage of the peritoneum, injection of gas into the abdominal space, damage of the vessels of the pelvis. This method helps to avoid adverse outcomes such as bleeding, pneumoperetoneum, decreasing of oxygen saturation. The method is explained on Figures 1,2,3.

Figure 1 - The location of trocars during extraperitoneal laparoscopic prostatectomy: 1, 5 – trocars of 10mm, 2, 3, 4 – trocars of 5 mm.



The patient's position is on the back with a trunk inclination of 30-450. 2 cm of cut is made along the midline 1 cm below the navel. The anterior leaf of aponeurosis is opened and the rectus abdominis muscle is diverted after that, a finger dissection is performed to access the space of Retzius. Then a balloon dissector is carried out in the direction of the pubic, in which up to 800 ml of gas is insufflated under visual control. After creating the workspace, the balloon dissector is removed. With the index finger, the peritoneum peels off and shifts to the lateral sides of the surgical field, and 4 working trocars are installed under the control of the index finger (Figure 2), palpating the lower epigastric vessels from the inside (Figure 3). After that an optical trocar is installed. The bladder is bluntly and acutely exfoliated from the anterior abdominal wall and pubic bone. The bladder is

Figure 2 - Trocars of 2, 3, 4, 5 are installed under the control of a finger.

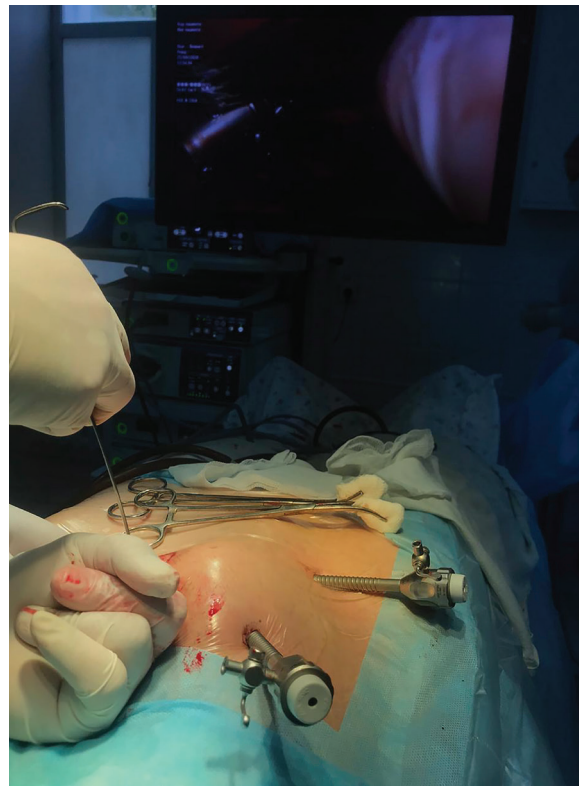


Figure 3 - Palpation of the lower epigastric vessels from the inside.

not enlarged. The prostate is visualized without signs of tumor germination into neighboring organs. The LigaSure apparatus gradually crosses the pubic-prostatic ligaments, between which the superficial branches of the dorsal vein of the penis (dorsal venous complex) are located, to the urethra. Capturing the tip of the prostate gland, Foley catheter No.16 is passed through it, inflate the cuff in the bladder. It is raised up, gradually peeling off the posterior surface of the prostate gland from the rectum. The lateral neurovascular bundle of the prostate gland is crossed by the LigaSure apparatus on both sides. When the posterior surface of the prostate gland and seminal vesicles are mobilized, the vessels feeding the seminal vesicles of the lateral walls of the prostate gland are ligated. The vas deferens intersects. Then the neck of the bladder is crossed, removing the prostate gland

and seminal vesicles in a single block, partially preserving the neck of the bladder, under the control of the estuaries of the ureters. Foley's catheter is passed through the urethra into the bladder, the cuff is inflated. The bladder is pulled up to the urethra; a single-row suture is applied to the urethrovesical anastomosis. Iliac lymphodissection is performed on both sides, as well as lymphodissection of the obturator fossa on both sides. Hemostasis is monitored. Hemostasis is dry; a retroperitoneal drainage tube is left in the pelvis, removed to the skin. Trocars are removed under the control of an endovideoscope. Layered suturing of the wound was carried out. An aseptic bandage was applied to the wound.

Conclusion

The main difference between the traditional laparoscopic procedure and our method is trocars insertion. During traditional laparoscopic procedure four working trocars are installed extraperitoneal under the control of optics. Our method recommends four working trocars are installed under the control of the index finger, palpating the lower epigastric vessels from the inside which allows avoiding bleeding, pneumoperitoneum, and decreased saturation; and in the early postoperative period, peritonitis is excluded and the late postoperative period, adhesive processes do not occur.

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Clinical and laboratory COVID-19 features in hospitalized patients with concomitant diabetes mellitus type 2: A retrospective study

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Abstract

Objective: To investigate the prevalence of diabetes mellitus in comorbidity structures and its effect on the clinical course in hospitalized COVID-19 patients in south region of Kazakhstan.

Material and methods: A retrospective analysis of data from 918 patients with COVID-19 treated at the City Clinical Infectious Diseases Hospital was carried out. Pearson's Chi-square test and Student's t-test were conducted.

Results: In Kazakhstan, diabetes mellitus occupies the second position in the structure of comorbidities in patients with COVID-19 with a share of 20%. Diabetes mellitus in patients most often occurs in combination with cardiovascular diseases and arterial hypertension (20.3% and 16.3%, respectively). Combination of diabetes mellitus, arterial hypertension and other diseases was detected in 72.4% of patients. Combination of diabetes mellitus, cardiovascular and other diseases was detected in 32.5%.

In diabetes mellitus patients, COVID-19 was more severe, the hospital stay was longer, and patients over 60 years of age suffered. These patients had a combination of diabetes mellitus with arterial hypertension, obesity, and cardiovascular diseases. Hyperglycemia, elevated blood pressure, rapid breathing, and low saturation were more common for these patients.

Conclusion: Diabetes mellitus ranks second in the structure of comorbidities in COVID-19 in the south region of Kazakhstan and is characterized by a combination with cardiovascular diseases, arterial hypertension and obesity. In patients with diabetes, COVID-19 is more severe, which affects the length of stay in the hospital, the mortality rate and the need for transfer to the Intensive Care Unit.

Key words: pandemic, COVID-19, diabetes mellitus, comorbidities

Introduction

The disease, first registered in Wuhan (China) in 2019, caused by SARS-CoV-2 and named by the World Health Organization (WHO) as COVID-19, led to the development of a pandemic and caused enormous damage to the global community [1]. By September 2022, about 598 million cases with about 6.5 million deaths had been detected worldwide [2]. The COVID-19 epidemic in Kazakhstan, as well as throughout the world, was fluctuating in nature with a sharp increase in the first half of 2020 [3] with higher mortality in PCR-positive patients [4].

To date, a meta-analysis of data has shown that diabetes mellitus (DM) is one of the three most common

comorbidities in COVID-19 [5,6]. Previous studies in Kazakhstan showed a similar situation with diabetes mellitus [7]. At the same time, the proportion of diabetes mellitus in the structure of comorbidities differs somewhat by region [6].

The impact of diabetes on the formation of a more severe course of COVID-19 and mortality rates was also revealed [8-11]. In particular, Varikasuvu S.R. et al. showed that the risk of severe COVID-19 in patients with diabetes is 2.2 times higher than in non-diabetic individuals, while mortality is 2.5 times higher [10]. At the same time, the intensity of this influence also varies depending on the geographical region [6]. Thus, it is relevant to analyze the structure of comorbidities and assess their impact on the severity and mortality of COVID-19 in certain regions, which will contribute to the development of a more effective strategy for the rehabilitation of people who have had COVID-19 in a particular region. With regard to DM, this is the most appropriate, since, according to studies by Bhaskar Thakur et al. [6], there is no direct correlation between the incidence of diabetes mellitus and the mortality rate from COVID-19 by region.

In Kazakhstan, at the moment there is an unfavorable situation for diabetes mellitus. In particular, at the beginning of 2021, 382000 people were registered at the dispensary for «diabetes mellitus». For 15 years, the incidence of type 2 diabetes has increased by 3.5 times [12].

Thus, we believe that studying the impact of diabetes mellitus on the course of COVID-19 in Kazakhstan will contribute to the development of a personalized approach to the prevention and treatment of COVID-19 in patients with type 2 diabetes mellitus.

Purpose of the study: To investigate the prevalence of diabetes mellitus in comorbidity structures and its effect on the clinical course in hospitalized COVID-19 patients in south region of Kazakhstan

Material and methods

Participants

A retrospective case-control study was conducted. We studied the 918 COVID-19 patients case histories treated at the City Clinical Zhekenova Infectious Diseases Hospital in Almaty in 2020-2021. In all patients, the COVID-19 diagnosis was confirmed by PCR. PCR was performed by quantitative real-time PCR on nasopharyngeal swabs using the BGI kit (Beijing Genomics Institute, Shenzhen, China) in accordance with the manufacturer's instructions.

The study was approved by the Ethics Committee of the Asfendiyarov Kazakh National Medical University (No.1293, 01/26/2022). Inclusion Criteria: All hospitalized COVID-19 patients. The clinic administration is informed, the clinic employees took part in the study, and there are no objections to the publication of data in the open press.

Inclusion Criteria: Participants were included in the study based on the protocol (National Clinical Protocol: "Coronavirus Infection COVID-19 in Adults" No.1. dated 03/02/2020) [12].

Exclusion criterion: persons not included in the protocol.

Data collection

Hospitalization indications

- Moderate Covid-19 infection: patients with following risk factors, symptoms and vital signs. Risk factors are age over 60 years, diabetes, hypertension, etc.; Respiratory rate (RR) 20-24, SpO2 - 93-95%, lung damage volume above 25%.

- Extremely severe or critical severity of COVID-19 (formation of acute respiratory distress syndrome, sepsis, septic shock, etc.)

- Patients with a fever of 380C and above for 3 days, resistant to antipyretic drugs.

- RR >24; increasing shortness of breath during normal household stress, talking; decrease in SpO2 <93%.

Clinical data included life and disease history, results of objective examination.

The study included individuals with a history of diabetes mellitus, who were registered with specialists at the dispensary.

Data analysis

The obtained results were processed in the IBM SPSS Statistics 17 statistical package. The following variables were used for the analysis: qualitative (disease history, presence of concomitant diseases, gender) and quantitative (age, length of stay in the hospital, hospitalization time from the onset of symptoms, clinical and laboratory values). Quantitative and qualitative data were shown using descriptive statistics. Comparison of the two groups by qualitative characteristics was performed by using Pearson's Chi-square test. To compare several groups at the same time, we used the $\chi 2$ test for arbitrary tables. Comparison of quantitative data was conducted using Student's t-test for unrelated variables. Differences were considered statistically significant at $p \leq 0.05$.

Results

Study population

The study population is presented in Table 1.

All patients with COVID-19 were over 18 years of age. The average age of patients with coronavirus infection was 54.4±17.02 years. There were 43% men and 57% women in the study group. A history of lung disease, hypertension, diabetes mellitus and obesity were noted in 10,5%, 39,4%, 13,4% and 6,2%, respectively. Leukopenia was detected in 238 (25,9%) patients, lymphopenia in 408 (44,4%) and thrombocytopenia in 311 (33,9%) patients, while 75 patients had leukocytosis, 31 had lymphocytosis and 88 had thrombocytosis (8,2%, 3,4% and 9,6% respectively). Low fibrinogen level was noted in 413 (45%) patients. The number of patients with a temperature above 38°C on admission was 116 (12,6%), while subfebrile temperature was noted in 802 (87,4%) patients. A normal level of systolic blood pressure was detected in 845 (92.1%) patients. Pneumonia on admission was noted in 639 (69.6%) patients. Deceleration of the frequency of RR and HR was detected in 795 (86.6%) and 18 (2%) patients, respectively. An increase in the number of heartbeats was noted in 114 (12,4%) patients. Patients with a low saturation level accounted for 34,3%.

The results of the analysis of the comorbidities structure COVID-19 patients are presented in Table 2. The number of patients with comorbidities was 609 (66%), while 309 (34%) patients did not have any. Arterial hypertension (AH) (59.4%), diabetes mellitus (DM) (20%), and cardiovascular diseases (CVD) (18.5%) occupy leading positions in the structure of comorbidities. In addition, gastrointestinal diseases (GD) (16.4%), bronchitis (10.8%), obesity (9.3%), anemia (3.4%), chronic kidney disease (1.8%) and other diseases (28.2%), which include thyroid diseases, rheumatoid diseases, eye diseases, autoimmune diseases, diseases of the upper respiratory tract, etc. have been identified. The structure of comorbidities in patients with COVID-19 is shown in Figure 1.

Our analysis of the comorbidities structure in patients with COVID-19 also showed that DM is most common in combination with CVD and AH (20.3% and 16.3%, respectively) (Table 3).

Table 1 Characteristics of patients with COVID-19

Parameters		Patients with COVID-19 N (%)
Total		918 (100%)
Sex	Male	395 (43%)
	Female	523 (57%)
Age (years) M ± m		54,4±17,02
Having a history of lung disease, n (%)	Yes	96 (10,5%)
	No	822 (89,5%)
Having a history of hypertension, n (%)	Yes	362 (39,4%)
	No	556 (60,6%)
Having a history of diabetes, n (%)	Yes	123 (13,4%)
	No	795 (86,6%)
Presence of obesity, n (%)	Yes	57 (6,2%)
	No	861 (93,8%)
Leukocytes, n (%)	Up to 3,9x109/l	238 (25,9%)
	4 - 9x109/l	605 (65,9%)
	Over 9,1x109/l	75 (8,2%)
Lymphocytes, n (%)	Up to 0,9x109/l	408 (44,4%)
	1,0-3,2x109/l	479 (52,2%)
	Over 3,3x109/l	31 (3,4%)
Fibrinogen, n (%)	Up to 4 g/l	413 (45%)
	Over 4,1g/l	505 (55%)
Platelets, n (%)	Up to 179 x 109/l	311 (33,9%)
	180-320x 109/l	519 (56,5%)
	Over 321x109/l	88 (9,6%)
Temperature at admission, n (%)	<38°C	802 (87,4%)
	>38°C	116 (12,6%)
Systolic blood pressure on admission, mmHg, n (%)	Up to 139	845 (92,1%)
	140 -160	47 (5,1%)
	Over 160	26 (2,8%)
Presence of pneumonia on admission, n (%)	Yes	639 (69,6%)
	No	279 (30,4%)
RR*, n (%)	Below 20	795 (86,6%)
	Over 21	123 (13,4%)
HR**, n (%)	Up to 59	18 (2%)
	60-100	786 (85,6%)
	Over 101	114 (12,4%)
Saturation on admission, n (%)	Below 94%	315 (34,3%)
	Over 95%	603 (65,7%)

Abbreviation:
RR* – respiratory rate
HR** – heart rate

Figure 1 - The structure of comorbidities in patients with COVID-19

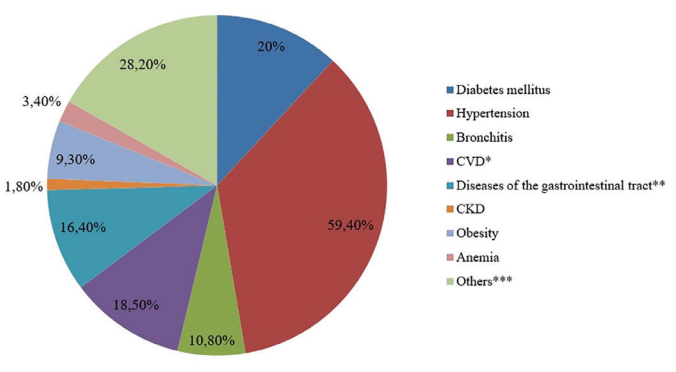

Table 2 The structure of comorbidities in patients hospitalized with COVID-19

Total - 918			
Of which:	N	%	
No comorbidities	309	34	
There are comorbidities	609	% of total	% of comorbidities
Of which:			
Diabetes mellitus	123	13,3	
	20,0		
Hypertension	362	39,4	
	59,4		
Bronchitis	66	7,1	10,8
CVD*	113	12,3	18,5
Diseases of the gastrointestinal tract**	100	10,8	16,4
CKD	11	1,1	1,8
Obesity	57	6,2	
	9,3		
Anemia	23	2,5	
	3,4		
Others***	172	18,7	28,2

Abbreviation:
CVD - cardiovascular diseases
GIT - gastrointestinal tract
CKD - chronic kidney disease
**CVD - ischemic heart disease, angina pectoris, cardiac arrhythmias
**Diseases of the gastrointestinal tract - stomach ulcers, gastritis, pancreatitis, cholecystitis, colitis, enteritis, hepatosis, hepatitis
***Others - thyroid disease, rheumatoid disease, eye disease, autoimmune disease, upper respiratory tract disease, etc.

Table 3 The structure of comorbidities in patients of diabetes mellitus having COVID-19

Parameters	Amount, n	%
The total number of patients with diabetes mellitus in combination of all concomitant diseases	123	100
In which:		
Diabetes mellitus (isolated)	12	9,8
Presence of diabetes mellitus + hypertension	20	16,3
Presence of diabetes mellitus + hypertension + other diseases	89	72,4
Presence of diabetes mellitus + lung disease	4	3,3
Presence of diabetes mellitus + lung diseases + other diseases	15	12,2
Presence of diabetes + obesity	4	3,3
Presence of diabetes mellitus + obesity + other diseases	13	10,6
Presence of diabetes mellitus + CVD	25	20,3
Presence of diabetes mellitus + CVD + other diseases	40	32,5
Presence of diabetes mellitus + gastrointestinal diseases	9	7,3
Presence of diabetes mellitus + gastrointestinal diseases + other diseases	13	10,6
Presence of diabetes mellitus + other diseases*	26	21,1

*Others - thyroid disease, rheumatoid disease, eye disease, autoimmune disease, upper respiratory disease, etc.

At the same time, the presence of DM, AH and other diseases was detected in 72.4% of patients, and the number of patients with a combination of diabetes mellitus, cardiovascular and other diseases was 32.5%. The number of patients with only DM was identified in only 9.8% of patients. The combination of DM with gastrointestinal diseases was detected in 7.3% of patients. At the same time, the number of patients with diabetes, gastrointestinal disease and other diseases was 10.6%. The combination of DM with lung diseases, as well as the combination of DM with

Table 4 Comparative characteristics of demographic and clinical-laboratory parameters of COVID-19 patients with and without diabetes mellitus

Parameters	DM absence (n=795)	DM presence	p (n=123)
Indicators			
Demographic and general clinical indicators			
Sex, n (%)			
Female	445 (56%)	78 (63%)	0,121
Male	350 (44%)	45 (37%)	
Age, n (%)			
18 to 44 years old	277 (34,8%)	8 (6,5%)	<0,001
45 to 59 years old	189 (23,8%)	26 (21,1%)	
60 and older	329 (41,4%)	89 (72,4%)	
Hospitalization time from the onset of symptoms, n (%)			
Until 10 days	661 (83,1%)	94 (76,4%)	0,070
More than 10 days	134 (16,9%)	29 (23,6%)	
The course of the disease, n (%)			
Severe	171 (22%)	59 (48%)	<0,001
Medium severity	624 (78%)	64 (52%)	
Lethal outcome, n (%)			
Yes	6 (1%)	5 (4%)	0,002
No	789 (99%)	118 (96%)	
Length of stay in hospital (bed days), n (%)			
Until 10 days	631 (79,4%)	70 (56,9%)	<0,001
More than 10 days	164 (20,6%)	53 (43,1%)	
Transfer to the intensive care unit, n (%)			
Yes	10 (1,3%)	12 (9,8%)	<0,001
No	785 (98,7%)	111 (90,2%)	
Comorbidities			
Hypertension, n (%)			
Yes	272 (34%)	90 (73%)	<0,001
No	523 (66%)	33 (27%)	
Having a history of lung disease, n (%)			
Yes	79 (10%)	17 (14%)	0,191
No	716 (90%)	106 (86%)	
Presence of obesity, n (%)			
Yes	41 (5%)	16 (13%)	<0,001
No	754 (95%)	107 (87%)	
A history of kidney disease, n (%)			
Yes	10 (1%)	4 (3%)	0,094
No	785 (99%)	119 (97%)	
The presence of diseases of the gastrointestinal tract in history, n (%)			
Yes	106 (13,3%)	13 (10,6%)	0,396
No	689 (86,7%)	110 (89,4%)	
Having a history of CVD, n (%)			
Yes	73 (9,2%)	40 (32,5%)	<0,001
No	722 (90,8%)	83 (67,5%)	
Having a history of anemia, n (%)			
Yes	18 (2,3%)	6 (4,9%)	0,091
No	777 (97,7%)	117 (95,1%)	
Presence of other diseases in history, n (%)			
Yes	110 (13,8)	16 (13%)	0,804
No	685 (86,2%)	107 (87%)	
Laboratory indicators			
The level of glucose in the blood at the time of admission, n (%)			
<4,0 mmol\l	31 (4%)	3 (2,5%)	p<0,001
4,1-6,1 mmol\l	559 (70%)	40 (32,5%)	
>6,2 mmol\l	205 (26%)	80 (65%)	
Leukocytes, n (%)			
Up to 3,9x109/l	210 (26%)	28 (23%)	0,097
4 - 9x109/l	526 (66%)	79 (64%)	
Over 9,1x109/l	59 (8%)	16 (13%)	
Lymphocytes, n (%)			
Up to 0,9x109/l	359 (45%)	49 (40%)	0,390
1,0-3,2x109/l	408 (51%)	71 (58%)	
Over 3,3x109/l	28 (4%)	3 (2%)	
Fibrinogen, n (%)			
Up to 4 g/l	363 (46%)	50 (41%)	0,299
Over 4,1 g/l	432 (54%)	73 (59%)	
Platelets, n (%)			
Up to 179 x 109/l	260 (33%)	51 (42%)	0,079
180-320x 109/l	461 (58%)	58 (47%)	
Over 321x109/l	74 (9%)	14 (11%)	
Activated partial thromboplastin time, n (%)			
Up to 24 sec	25 (3%)	1 (1%)	0,226
25-35 sec	497 (63%)	84 (68%)	
Over 36 sec	273 (34%)	38 (31%)	
Prothrombin index, n (%)			
Up to 79 %	134 (17%)	19 (15%)	0,803
80-100%	590 (74%)	91 (74%)	
Over 101%	71 (9%)	13 (11%)	
Data of an objective clinical and instrumental study			
Temperature at admission, n (%)			
<38°C	693 (87%)	109 (89%)	0,653
>38°C	102 (13%)	14 (11%)	
Blood pressure on admission, n (%)			
Up to 139 mmHg	733 (92%)	112 (91%)	0,002
140-159 mmHg	45 (6%)	2 (2%)	
Over 160 mmHg	17 (2%)	9 (7%)	
Pneumonia, n (%)			
Yes	549 (69%)	90 (73%)	0,356
No	246 (31%)	33 (27%)	
Respiratory rate per minute on admission, n (%)			
16-20	696 (87,5%)	96 (78%)	0,015
Less than 15	2 (0,3%)	1 (1%)	
More than 21	97 (12,2%)	26 (21%)	
Heart rate per minute on admission, n (%)			
Up to 59	16 (2%)	2 (2%)	0,536
60-100	684 (86%)	102 (83%)	
More 101	95 (12%)	19 (15%)	
Saturation on admission, n (%)			
Up to 94	251 (32%)	64 (52%)	p<0,001
More 95	544 (68%)	59 (48%)	

obesity, was the same (3.3%). At the same time, the number of patients with diabetes, lung diseases and other diseases, as well as the number of patients with diabetes, obesity and other diseases was 12.2% and 10.6%, respectively.

A comparative analysis of the demographic and clinical and laboratory characteristics of COVID-19 patients with and without diabetes mellitus showed a number of significant differences between the indicators (Table 4).

Thus, among patients with diabetes, persons over the age of 60 met 1.7 times more often compared with persons not suffering from DM (72,4% and 41,4%, respectively). The severe course of the COVID-19 in patients with DM was observed 2.18 times more often than in patients without DM (48% versus 22%). Fatal outcome was also 4 times more common among diabetic patients (4% vs. 1%). The presence of arterial hypertension in history in patients with DM was noted 2.14 times more often (73% vs. 34%), while an increased level of blood pressure at admission in patients with DM was registered 7 times more often (7% vs. 1%). In addition, the presence of cardiovascular diseases in patients with diabetes was detected 3.5 times more often (32.5% versus 9.2%). Obesity occurred in DM patients 2.6 times more often (13% versus 5%). The presence of kidney pathology in history in patients with DM was noted 3 times more often, however, the significance was not revealed.

As for laboratory parameters, significant differences were noted only in the level of glucose in the blood at the time of admission. In patients with diabetes mellitus, the glucose level n the blood above the norm was observed 2.5 times more often than in patients without diabetes (65% vs. 26%).

According to the indicators of clinical and instrumental studies, there were significant differences in the RR and the saturation level at admission. Thus, the saturation level below 94% in diabetic patients was observed 1.62 times more often than in patients without diabetes (52% vs. 32%), and RR above 21 in diabetic patients was recorded 1.72 times more often (21% versus 12.2%).

Discussion

The results of the analysis of the comorbidities structure in COVID-19 patients in our study showed that 66% of COVID-19 hospitalized patients had comorbidities. Previously, Hui Poh Goh et al showed the structure of comorbidities in COVID-19 in different regions of the world. The share of diabetes in the comorbidities structure in COVID-19 was 31% in the USA, 20% in Europe, 18% in Latin America and 14% in Asia [6]. In our study, diabetes mellitus is in second place (20%) after arterial hypertension (59.4%). Cardiovascular diseases are in third place (18.5%). This distribution of positions is consistent with the data for the Asian region [6].

At the same time, the proportion of diabetes mellitus in the structure was 20%, which is closer to the figures for the European Region (20%) and higher than in the Asian Region (14%), which causes concern.

An analysis of the comorbidities structure in DM patients showed that comorbidities were 1.4 times more common for DM patients compared to the general population of COVID-19 patients (90.2% vs. 66.0%). At the same time, diabetes mellitus was more often combined with arterial hypertension (72.4%), cardiovascular diseases (32.5%), lung diseases (12.2%), as well as obesity and gastrointestinal diseases (in equal combination in 10, 6% of cases).

It should be noted that all these diseases associated with diabetes are independent risk factors for the formation of a severe COVID-19 [13-17].

Moreover, the combination of arterial hypertension, obesity and cardiovascular disease has been shown to be the main condition for the development of the metabolic syndrome, which can lead to severe COVID-19 [18-22].

Thus, it can be assumed that the previously identified role of diabetes mellitus in the development of severe COVID-19 [23-25] is due not only to the pathogenesis of diabetes mellitus [26-28], but also the presence of a large number of diseases associated with diabetes, which increase the impact on the severity of COVID-19.

We also found a wide range of differences in the characteristics of hospitalized COVID-19 patients with and without diabetes mellitus, confirming the role of diabetes in the formation of severe COVID-19. Thus, among COVID-19 patients, DM people over 60 years of age accounted for almost half of the total number of patients, which is associated with the characteristics of type 2 diabetes mellitus that mainly occurred in old age [29, 30].

According to our data, in patients with concomitant DM, COVID-19 was more severe, and a lethal outcome was noted more often than in its absence. In addition, according to the results of the analysis, it was revealed that patients with diabetes mellitus stayed in the hospital longer than patients without it.

Also, patients with diabetes mellitus were much more likely to have high blood pressure on admission. As noted above, this is because COVID-19 often has a combination of diabetes mellitus, hypertension, and cardiovascular disease. Also, patients with diabetes mellitus were much more likely to have high blood pressure on admission. As noted above, this is because COVID-19 often has a combination of diabetes mellitus, hypertension, and cardiovascular disease [18-22].

It is interesting that in the absence of differences in the incidence of pneumonia at admission in groups of patients with and without diabetes mellitus, dyspnea was more common in patients with diabetes mellitus and the saturation level was significantly lower. We believe that this is related with the more severe nature of lung damage in diabetic patients due to the presence of angiopathy with increased vascular permeability and collapse of the alveolar epithelium. On the other hand, in DM, there is usually a significant decrease in forced vital capacity and forced expiratory volume in one second, which is associated with increased plasma glucose levels [31].

The limitation of this study is the use of the results obtained in one hospital, and therefore additional studies are needed to objectively assess the situation in Kazakhstan.

Conclusion

The results of our retrospective analysis confirm the role of diabetes mellitus in the development of severe COVID-19. A high degree of combination of concomitant diabetes mellitus in patients with COVID-19 with other diseases was shown, which has a synergistic aggravating effect on the course of COVID-19. Our statistical analysis of the incidence of diabetes mellitus in the structure of comorbidities in hospitalized patients with COVID-19 in the southern region of Kazakhstan, as well as data on the structure and combination of comorbid pathology in patients with diabetes mellitus, will help optimize the treatment and rehabilitation of this category of patients.

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Effect of women's health literacy levels on their beliefs about breast cancer screening

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Abstract

Objective: This study was conducted to examine the effect of women's health literacy level on breast cancer screening beliefs.

Material and methods: This study has been done descriptively. In data collection, measurement tools such as 'Descriptive Information Form', 'Health Literacy Scale' and 'Breast Cancer Screening Beliefs Scale' developed based on literature and observations were used. Statistical analysis of the data was made in SPSS 20.0 statistical package program. Before the study was conducted, approval was obtained from XXX Faculty of Nursing Ethical Council.

Results: When the descriptive-variable characteristics of the women participating in the study were examined, it was determined that the difference between health literacy levels and breast cancer screening beliefs was significant ($p<0.05$).

Conclusion: As a result of the research, it was determined that as the health literacy level of women increased, the level of breast cancer screening beliefs also increased.

Key words: belief, breast cancer, breast cancer screening, health literacy

Introduction

Health services are needed for mental and bodily health, which is the common desire of whole humanity. Hence, everyone is a health service user. In this regard, they should know about their health as well as the health services they will use. Health literacy (HL) has an important place in individuals' access to and use of health services [1]. The WHO defines health literacy as the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions [2]. Although HL is of particular concern for all people, it affects women more because women's health could have positive or negative consequences on both their own health and the health of their families. Healthy women create healthy families, healthy families create healthy societies, and healthy societies create environments with high health and wealth levels. Many factors affect the HL level, which include population structure, cultural features, psychosocial factors, literacy level, individual characteristics, disease-related experiences, and financial resources of the country [3].

In their large-scale study entitled "Health literacy in Europe: comparative results of the European HL survey" and conducted in 2011, Sorensen et al. (2012) included eight European countries and found that the prevalence of insufficient HL was 47% [4]. In their society-based study entitled "Turkish HL Investigation", Tanrıöver et al. (2014) reported the insufficient HL as 64.6% (inadequate HL prevalence: 24.5%, problematic HL prevalence: 40.1%), and around two out of three people were reported to have insufficient HL level [5].

Inadequate/low HL level is reported to cause individuals to experience inadequacies in accessing, obtaining, understanding, and interpreting health-related information; have difficulties in understanding health-related problems and inadequacies in implementing treatments when they have problems about health; experience difficulties in performing medical procedures and instructions; have increases in the rates of applications to hospitals and hospitalizations; and demonstrate insufficient protection behaviors [6].

Together with the inadequacies in the use of protective health services, low HL causes restrictions

in screenings that have vital importance in the early diagnosis of diseases like cancer [7]. In other words, low/inadequate HL increases the use of health services for treatment purposes rather than protective health services. The use of health services for treatment purposes causes individuals to go to the hospital only when they have a disease, increases hospitalization rates, and decreases compliance with the treatment and satisfaction with health services [8]. Compared with women with marginal and adequate literacy, women with low literacy were significantly more likely to have negative attitudes about mammography including that a mammogram would be embarrassing, harmful, or painful, and were also more likely to feel that it would be a lot of trouble to get a mammogram [9]. Given the increase in its prevalence, early diagnosis and screening programs for breast cancers have vital importance in society [10]. Screening methods with proven effects are used for the early diagnosis of breast cancer; BSE (Breast Self-exam), CBE (Clinical Breast Exam) and mammography are among the screening methods used [11]. Diagnosing this public health problem early via breast cancer screening and minimizing the mortality rate could be possible by increasing the health knowledge and HL level. Recent studies have investigated the association of health literacy with cancer-related attitudes, knowledge, and behaviors to educate and increase patient trust, self-efficacy, and engagement in decision making. [9,12].

Women, who affect the majority of society as mothers and wives, should be helped to increase their knowledge and awareness about HL and provided with trainings on this issue, which is believed to contribute to positive developments in the HL level of the family, society, and country. As knowledge also brings belief, health knowledge level that increases women's HL also increases belief levels about being healthy. To what extent women, whose HL level is determined, believe, and attach importance to breast cancer and breast cancer screening could also be investigated.

Material and methods

Aim and type of the study

This descriptive study aims to determine women's health literacy level and its effects on their beliefs about breast cancer screening.

Population and sample of the study

The number of individuals to be included in the sample was determined using priori power analysis, and Cohen's standard effect sizes were taken as reference in the power analysis performed [13]. The minimum sample size to represent the population was calculated as 385 at 95% confidence interval. Considering potential data loss, extra 10% of sample was added to this number, and the study was completed with 400 women.

Place and time of the study

Data were collected between the 1st of May and the 30th of June 2021. The study included women who lived in Van province, who were at least literate, who were aged below 65, who did not have a psychiatric problem, who did not have a hearing problem, who could communicate sufficiently, and who agreed to participate in the study.

Data collection tools

Data were collected through the "Personal Information Form", the "Health Literacy Scale" and the ''Breast Cancer Screening Beliefs Questionnaire".

The Personal Information Form: The form was prepared by the researchers in line with the literature and included questions to collect data about women's socio-demographic characteristics, characteristics of spouses in married women, characteristics about reading books, and knowledge about breast cancer and breast cancer screenings.

The Health Literacy Scale: The scale was developed by Suka et al. (2013) in Japan in 2010 to measure adults' HL levels [14]. Turkish adaptation and reliability and validity of the scale were performed by Türkoğlu and Kılıç in 2021 [15]. The scale has three sub-scales including Functional HL (5 items), Interactive HL (5 items), and Critical HL (4 items). Cronbach's alpha value of the original scale was found 0.81. This study found Cronbach's alpha value as 0.94, and Cronbach's alpha values of the sub-scales ranged between 0.86 and 0.92.

Breast Cancer Screening Beliefs Questionnaire: The scale was developed by Kwok et al. (2010) in 2010 to determine women's breast cancer screening beliefs [16]. Turkish adaptation, reliability, and validity of the scale were performed by Türkoğlu and Sis Çelik in 2021 [17]. The scale has three sub-scales including Attitudes towards General Health Checkups, Knowledge and Perceptions about Breast Cancer, and Perceived Barriers to Mammographic Screening. Cronbach's alpha internal coefficients were found to range between 0.76 and 0.87 in the sub-scales of the original scale. The scores to be obtained from the scale range between 0 and 100. Mean scores of 65 and over in the sub-scales indicate that screening beliefs increase positively, knowledge level increases, and barriers to mammography screening decrease. This study found Cronbach's alpha value as 0.77.

Data Collection: Data were collected between the 1st of May and the 30th of June 2021 from women who agreed to participate in the study after their verbal consent was received. The online questionnaires prepared in Google forms and the scale questions were sent to the participants through WhatsApp on their phones, and data were collected through the snowball sampling method.

Data Analysis: Data were analyzed in SPSS 20.0 statistical package program. Analyses included t-test in independent groups, one-way analysis of variance (ANOVA), Kruskal Wallis test, and Mann Whitney-U test. Descriptive values included numbers and percentages in categorical data and arithmetic means and standard deviation values in quantitative data. The statistical significance level was accepted $p<0.05$.

Ethical considerations

Before the study was conducted, approval was obtained from XXX Faculty of Nursing Ethical Council on April 5, 2021 (Approval no. 2021-1/9). Participants of the study were informed that their personal information would not be disclosed to any person other than the researcher, and that no one else would be able to access the information.

Results

Of all the participating women, 53.75% were aged 18 to 30, 57.75% had an education level of university and above, 53.75% were married, 80.75% had a nuclear family, 57.25% worked, 57.5% had a medium economic level, and 76.25% did not have a chronic disease. Besides, 81% read books, 64.5% liked reading written materials, and 81.75% defined their general health condition as good. It was also found that 57.1% of the spouses of married women were aged between 20 and 40 and 68.2% had an education level of university and above. Of all the participating women, 69.5% received information about breast

Table 1 Sociodemographic of Women, Reading and Distribution of Characteristics of Health Conditions

	S	%	HL Total	Breast Cancer Screening Beliefs Total
Age				
18-30	215	53.7	47.18±3.92	71.94±15.28 a
31-40	98	24.5	46.92±3.77	69.38±15.89 a
41-50	48	12	47.83±3.73	66.46±15.91 ab
51 and above	39	9.7	47.64±2.82	62.32±21.08 b
Statistical Analysis			F:0.798 p:0.496	F:4.742 p:0.003
Level of Education				
Literate	21	5.25	46.81±4.85	37.82±8.41 d
Primary School Graduate	19	4.75	47.63±2.58	59.31±12.66 c
Secondary School Graduate	42	10.5	46.81±2.68	62.72±13.43 bc
High School Graduate	87	21.75	46.43±2.92	68.87±12.19 ab
University or Higher Graduate	231	57.75	47.63±4.13	75.06±14.79 a
Statistical Analysis			F:1.915 p:0.107	F:42.400 p:0.001
Marital Status				
Married	215	53.75	47.20±3.67	70.17±17.91
Single	185	46.25	47.29±3.88	69.18±14.41
Statistical Analysis			t:0.065 p:0.799	t:0.363 p:0.547
Family structure				
Core	323	80.75	47.43±3.88	71.65±15.30 a
Wide	56	5.25	46.25±3.28	60.37±18.15 b
Parents Separated	21		47.00±2.81	64.83±18.43 b
Statistical Analysis			F:2.389 p:0.093	F:13.071 p:0.001
Working Status				
Working	229	57.25	47.57±4.01	74.03±14.64
Not Working	17	42.75	46.80±3.36	63.94±16.82
Statistical Analysis			t:4.080 p:0.044	t:40.868 p:0.001
Economic Situation				
Good	156	39	47.22±3.58 a	74.93±14.09 a
Middle	230	57.5	47.36±3.72 a	66.98±16.24 b
Bad	14	3.5	45.43±5.93 b	56.59±23.89 c
Statistical Analysis			F:1.743 p:0.176	F:16.876 p:0.001
Chronic Disease				
Yes	95	23.75	47.08±3.14	65.14±19.38
No	305	76.25	47.29±3.94	71.14±15.07
Statistical Analysis			t:0.212 p:0.645	t:9.961 p:0.002
Reading Status				
Yes	324	81	47.33±3.82	73.40±14.39
No	76	19	46.86±3.50	53.99±15.01
Statistical Analysis			t:0.977 p:0.323	t:110.145 p:0.001
How She Evaluates Her General Health Status				
Very good	55	13.75	48.05±3.10	85.06±11.40 a
Good	327	81.75	47.14±3.87	68.14±15.10 b
Bad	18	4.5	46.50±3.46	51.49±18.56 c
Statistical Analysis			F:1.744 p:0.176	F:44.929 p:0.001

a, b, c... : The difference between groups that received different letters for each feature was statistically significant (p<0.05).

cancer and early diagnosis of breast cancer, 94.75% did not have anyone with breast cancer in their family or close relatives, 78.85% had heard about BSE (Breast Self-exam) before, 62% knew how to do BSE, and 50.5% did BSE regularly. It was found that 69.6% of women heard about CBE (Clinical Breast Exam) before, 85.2% heard about mammography before, 95.5% did not have mammography before, and 92.25% did not have mammary ultrasonography before.

The difference in the HL scale total mean score was found to be statistically significant according to women's working or not (p<0.05) (Table 1). Mean scores were found to be higher in women who worked and who liked reading a lot.

The difference between the breast cancer screening total score was found to be significant according to the characteristics such as women's age, education level, family structure, working or not, economic level, chronic disease, reading books, enjoying reading written materials, and perceived general health condition (p<0.05) (Table 1). Mean scores were found to be higher in women who were aged 18 to 30, who had an education level of university and above, who had a nuclear family structure, who worked, who had a good economic condition, who did not have a chronic disease, who read books, who liked reading a lot, and who defined their health as very good.

The difference in the HL scale total score was statistically significant according to women's having heard about BSE, CBE and mammography before and having had mammary ultrasonography before (p<0.05) (Table 2). The mean scores were found to be higher in women who heard about BSE, CBE, and mammography before and who had mammary ultrasonography before.

The difference in the breast cancer screening questionnaire total score was found to be significant according to the age and education level of spouses (p<0.05) (Table 2). Total mean scores were higher in women whose spouses were aged between 20 and 40 and whose spouses had an education level of university and above.

Table 2 Woman's Spouse and Pregnancy Status, Information Distribution on Breast Cancer and Early Diagnosis in Breast Cancer

	S	%	HL Total	Breast Cancer Screening Beliefs Total
Husband's Age				
20-40	122	57.1	46.86±3.96	72.96±17.45 a
41-60	76	35.5	47.58±3.23	67.51±17.46 ab
61 and above	16	7.4	47.94±3.45	61.53±20.43 b
Statistical Analysis			F:1.241 p:0.291	F:4.290 p:0.015
Spouse's Education Level				
Primary School Graduate	7	3.2	49.00±6.72	46.15±16.89
Secondary School Graduate	11	5.1	47.73±3.90	43.88±14.53
High School Graduate	51	23.5	47.53±2.73	62.78±16.30
University or Higher Graduate	148	68.2	46.99±3.80	75.76±15.17
Statistical Analysis			F:0.644 p:0.632	F:19.834 p:0.001
Do you know about breast cancer and its early detection?				
Yes	278	69.5	47.45±3.33	73.42±14.21
No	122	30.5	46.77±4.58	61.27±17.84
Statistical Analysis			t:2.735 p:0.099	t:52.810 p:0.001
Having a family or close relative with breast cancer				
Yes	23	5.25	48.23±2.63	76.73±18.99
No	377	94.75	47.18±3.82	69.30±16.16
Statistical Analysis			t:1.600 p:0.207	t:4.214 p:0.041
The state of hearing BSE before				
Yes	315	78.75	47.53±3.43	71.84±14.72
No	85	21.25	46.16±4.68	61.85±19.60
Statistical Analysis			t:8.959 p:0.003	t:26.492 p:0.001
The state of knowing how BSE is done				
Yes	248	62	47.50±3.34	74.34±14.40
No	152	38	46.82±4.35	62.15±16.62
Statistical Analysis			t:3.122 p:0.078	t:60.015 p:0.001
Condition of performing BSE regularly				
Yes	202	50.5	47.59±3.39	75.31±14.62
No	198	49.5	46.88±4.09	64.01±16.13
Statistical Analysis			t:3.523 p:0.061	t:53.908 p:0.001
The state of hearing CBE before				
Yes	278	69.6	47.59±3.26	73.04±14.71
No	122	30.5	46.44±4.64	62.15±17.48
Statistical Analysis			t:7.993 p:0.005	t:41.270 p:0.001
Having heard of mammography before				
Yes	343	85.2	47.55±3.59	71.05±15.20
No	57	14.8	45.39±4.25	61.70±20.58
Statistical Analysis			t:16.716 p:0.001	t:16.538 p:0.001
Have you had a mammogram before?				
Yes	18	4.5	48.58±3.74	72.17±16.13
No	382	95.5	47.17±3.76	69.61±16.39
Statistical Analysis			t:2.527 p:0.113	t:0.397 p:0.529
Having had a breast ultrasound				
Yes	31	7.75	48.67±3.63	74.18±15.98
No	369	92.25	47.11±3.75	69.31±16.37
Statistical Analysis			t:5.207 p:0.023	t:2.682 p:0.102

a, b, c... : The difference between groups that received different letters for each feature was statistically significant (p<0.05).

Table 3 The Health Literacy Scale and Breast Cancer Screening Beliefs Questionnaire Mean Scores

	Scale	Number of items	Min-max	X±SS
HL Scale	Functional HL sub-scale	5	5-25	19.81±4.36
	İnteractive HL sub-scale	5	5-25	20.44±3.43
	Critical HL sub-scale	4	6-20	16.61±2.28
	Total	**14**	**20-70**	**56.87±9.09**
Breast Cancer Screening Beliefs Questionnaire	Health Screening sub-scale	4	0-100	53.03±23.12
	Knowledge and Perceptions of Breast Cancer sub-scale	4	0-100	77.37±18.52
	Barriers to Mammography Screenings	5	0-100	76.95±17.52
	Total	13	0-100	69.72±16.37

Table 4 Correlation of Scale Score Means (n=400)

		1	2
(1) HL Scale	r	-	0.708
	p	-	0.001
(2) Breast Cancer Screening Beliefs Questionnaire	r	0.708	-
	p	0.001	-

The difference between breast cancer screening beliefs questionnaire total score was found to be significant according to women's receiving information about breast cancer and early diagnosis of breast cancer, having someone with breast cancer in the family and close relatives, having heard about BSE before, knowing how to do it, and doing it regularly and having heard about CBE and mammography before ($p<0.05$) (Table 2). Mean scores were found to be higher in women who received information before, who had someone with breast cancer in the family or close relatives, who heard about BSE before, knew how to do it, and did it regularly, and who heard about CBE and mammography before.

Women were found to receive 19.81±4.36 in the functional HL sub-scale, 20.44±3.43 in the interactive HL sub-scale, 16.61±2.28 in the critical HL sub-scale, and 56.87±9.90 in the total HL mean score.

Mean scores were found 53.03±23.12 for the attitudes towards health screening sub-scale, 77.37±18.52 for the knowledge and perceptions of breast cancer sub-scale, 76.95±17.52 for the mammography sub-scale, and 69.72±16.37 for the total breast cancer screening belief questionnaire (Table 3).

A positive, statistically significant, and medium-level relationship was detected between women's HL scale and breast cancer screening beliefs questionnaire mean scores ($p<0.05$). Women's breast cancer beliefs increase with the increase in their HL level (Table 4).

Discussion

This study found that younger women had higher breast cancer screening beliefs. It was reported that 79% of women who had just been diagnosed with breast cancer and 88% of women who died of breast cancer were aged 50 and above [18]. A study conducted with women in America defined breast cancer risks of women according to age as follows: risk until the age of 39: 0.49% (1 in 203 women), risk between the ages of 40 and 59: 3.76 % (1 in 27 women), risk between the ages of 60 and 69: 3.53% (1 in 28 women), and risk at the age of 70 and above: 6.58% (1 in 15 women) [12]. Percentages in these studies indicate that risk of having breast cancer and mortality rates associated with this increase demonstrates an increase with age. Hence, screening behaviors are expected to increase with the increase in the risk with aging; the lack of this increase is considered to be associated with the decrease in the HL level with aging.

This study found that women's breast cancer screening beliefs increased with the increase in their education level. Çidem and Ersin (2019) found that women's screening behaviors increased with the increase in the education level [19]. The study conducted by Duman et al. (2015) also found that regular BSE practice increased with the increase in the education level. This finding of the study is in line with the literature [20].

Women who had a nuclear family structure were found to have higher breast cancer screening beliefs compared to other groups. Pulgaron et al. (2016) reported that grandparents affected children's and grandchildren's receiving health services and increased the implementation of traditional health methods [21]. When this finding is considered, it seems that the presence of grandparents or mother or father-in-law is a barrier to benefiting from health services and distracts women from protective behaviors. Women in extended families have decreased beliefs about breast cancer screening, which is a protective behavior.

This study found that working women and women with good income levels had higher breast cancer screening beliefs. Regarding working and economic conditions, a study in the literature reported that individuals' support from social networks decreased the negative consequences of low knowledge levels [22]. This result suggests that the individual is within the family and social network, and understanding and using health information generally depends on others' knowledge and skills [23]. These studies indicate that working women are more conscious because their economic level is good and their social network is greater. Besides, higher breast cancer screening beliefs of working women and women with a good financial condition are considered to be associated with their easier access to health services and trainings.

This study found that women who did not have a chronic disease and whose perceived general health was good had higher breast cancer screening beliefs. A study conducted with individuals living in Japan reported that some patients with chronic diseases had low HL and knowledge scores [24]. Individuals with low HL were found to be less healthy, could cope with chronic diseases less, had less knowledge about health, and had difficulties in reading and understanding the information written in medicine leaflets or hospital forms [25]. The reason for lower HL levels in women with chronic diseases is considered to be associated with the increase in age-related chronic diseases and again age-related decrease in screening beliefs.

This study found that women who read books had higher breast cancer screening beliefs. Dişçigil et al. (2007) found that the rates of breast cancer screening behaviors increased directly proportionally with the education level [26]. This finding is considered to be associated with the increase in HL levels with the increase in reading and writing activities, which are considered to affect breast cancer knowledge levels and increase the belief levels.

The difference in the breast cancer screening questionnaire total score was found to be significant according to spouses' age and education level, and the women whose spouses were aged 20 to 40 and had an education level of university and above had higher breast cancer screening beliefs. Education and HL levels of people surrounding individuals affect their level of knowledge [27]. Family members with higher abilities could help other family members to perform health-related duties and have an independent contribution to the manageability of the health-related duties better [28]. The reason for the increase in women's health knowledge level due to their spouse's education level is considered to be associated with the increase in women's belief levels with the exchange of knowledge and beliefs about screenings in the family.

HL levels were found to be higher in women who heard about BSE, CBE, and mammography before and who had mammary ultrasonography before. Besides, breast cancer screening beliefs were found to be higher in women who received information about breast cancer and early diagnosis, who had someone with breast cancer in the family or close relatives, who heard about BSE before and knew how to do it, and who did it regularly, and who heard about CBE and mammography before. BSE, CBE, and mammography are highly important for the early diagnosis of breast cancer. Yılmazel (2013) found that performing BSE increased with the increase in women's education level [29]. Altunkan et al. (2008) reported an association between education level and performing BSE [30]. Considering the studies

conducted, screening behaviors used for the early diagnosis of breast cancer are directly proportional to literacy and education level. Women's awareness increased with the increase in their education level. Although women knew about breast cancer and early diagnosis of breast cancer, screening rates were found to be inadequate and low, indicating a lack of awareness about the importance of screenings.

A study conducted with women showed that the presence of a positive family history affected screening behaviors in women [31]. In this study, women's knowing someone with breast cancer seems to make them strive for learning more about health. With the information they gained, an increase was reported in their use of screening methods and belief levels, which is somewhat expected. A study on the experience and satisfaction of women who participated in breast cancer screenings showed that women generally reported high satisfaction with their screening experiences; their worries were eliminated; breast cancer screenings had relatively fewer effects on the social and physical aspects of their life but enabled positive effects on some emotional issues such as assurance, well-being, and relief [32]. Increase in screening beliefs with the increase in knowledge, conscious and education about early diagnosis of breast cancer is somewhat expected in women who had information about breast cancer screening behaviors and who had screenings.

Women, who form around half of society, are important parts of the family who have roles as mothers, wives, sisters, and daughters. The woman's age, education level, health-related beliefs, and access to health services are factors that affect their health [33]. HL level is one of the most important factors that affect women's health protection and improvement behaviors. This study found participating women's HL scale total mean score as 56.87±9.90, indicating an above-average HL level. Studies conducted worldwide indicate that HL is below expected levels [34,35]. A study conducted in the USA reported that 22% of adults' reading and writing skills remained at a basic level. The HL level determined with the TSOY-32 scale in our country indicated the HL levels as inadequate for 30.9%, problematic-insufficient for 38%, sufficient for 23.4%, and perfect for 7.7%; the study reported that 7 out of 10 people in our country had inadequate or insufficient HL level [36]. The same study reported that the prevalence of inadequate/low HL was 26.4% in males and 35.3% in females, indicating lower HL levels in women [5]. These findings of the related studies indicate that the HL level was higher in this study, which is considered to be caused by the different sample groups.

Women's breast cancer screening belief questionnaire mean score was found 69.72±16.37 in this study. Breast cancer is a serious health problem that affects women all over the world. Although it has a high mortality rate, death rates are reported to decrease with early diagnosis and treatment [37]. Low/inadequate HL is an important barrier to the early diagnosis and screening of breast cancer [38].

This study found that the difference between the HL scale and breast cancer screening beliefs scale was positive, medium-level, and statistically significant. Women's breast cancer screening beliefs increased with the increase in their HL level. In the study conducted with women aged 20 and over, Erbil and Bölükbaş (2012) found that self-efficacy and health motivation scores increased significantly with the increase in the education level [39]. This study indicated that the HL level increased with the increase in the education level, and demand and health-protecting and improving behaviors increased with the increase in HL.

Conclusion

Women's socio-demographic and breast health-related characteristics were found to affect their breast cancer screening behaviors. Besides, breast cancer screening beliefs increased with the increase in women's HL levels. Increased HL levels in women also increased the demand for screening methods that are important for early diagnosis of breast cancer, which is common in women; the increase in beliefs in screening is somewhat expected since cancer treatment is more effective with early diagnosis. It is believed that HL should be improved particularly in women with lower HL levels.

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Clinical management of cementifying fibroma: A case report and pertinent review of the current literature

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Abstract

Cementifying fibroma is an uncommon neoplasm composed by varying amounts of cementum, bone and fibrous tissue. As a results of having similar histolological features based on inactive-looking odontogenic epithelium embedded in a fibrous stroma, it is often hard to differentiate from other fibro-osseous lesions such as fibrous dyplasia and calcifying odontogenic tumor. At this point, it is undoubtedly clear that proper radiological and clinical diagnosis play a great role together in identification. We present a rare case of this entity along with a number of clinical and radiographic features that set it apart from other pathologies mimicking fibro-osseous lesions.

Key words: cemento-ossifying fibroma, odontogenic tumor, neoplasms, cone-beam computed tomography, pathology

Introduction

Fibro-osseous lesions (FOLs) were first included as a lesion group among odontogenic and maxillofacial bone tumors in the *Classification of Head and Neck Tumours*, published by the World Health Organization (WHO) in 2017. FOLs of the craniofacial complex comprise a subgroup of benign tumors that differ morphologically, clinically, and radiographically. There are three recognized types of FOLs; fibrous dysplasia (FD), cemento-ossifying fibroma (COF), and cemento-osseous dysplasia (COD). While FD can occur anywhere in the skeleton, COF and COD are found solely in the maxillofacial bones [1].

Cemento-ossifying fibroma (COF) is uncommon, benign, mesenchymal odontogenic tumors growing slowly out of the periodontal ligament, comprising of a layer of fibrous connective tissue that encircles the root part of the tooth [2]. The lesion is characterized by multipotential cells that are prone to forming cementum next to lamellar bone and fibrous tissues. Histologically, it exhibits variable quantities of cement clusters embedded in the fibrous tissue with regions of splintered and disorganized bone fragments [3].

Reported and prevalence series data indicate that women specifically aged between 30 and 40 years are more likely to have the lesions than men. COF is more common in the mandible (70% frequency) than in the maxilla and posterior regions are reportedly exposed to higher risks than anterior ones [3]. Clinicians occasionally identify a lesion via an orthopantomogram during a routine dental checkup. The lesions can be defined with various degrees of opacification depending on multilocular or unilocular mixed radiolucent and radiopaque masses with marginal sclerosis [4].

A COF is generally a spherical or egg-shaped, slowly expanding mass that affects the teeth-bearing areas, resulting in root resorption of varying degrees on adjacent teeth. Although the origin of COF is still debated, it is considered that traumatic injuries or local irritants are possible reasons for their occurrence [5]. In the present study, we discuss the case of a 23-year-old male who was referred to the oral surgery department just before he was about to be conscripted into the military. The report also aims to discuss COF as a distinct type of ossifying fibroma and rare tumor and conduct a literature review on the current discussion about whether a new category of ossifying fibroma of non-odontogenic origin should be designed. The objective of this case report was to guide the clinicians in diagnosing and managing of a COF case.

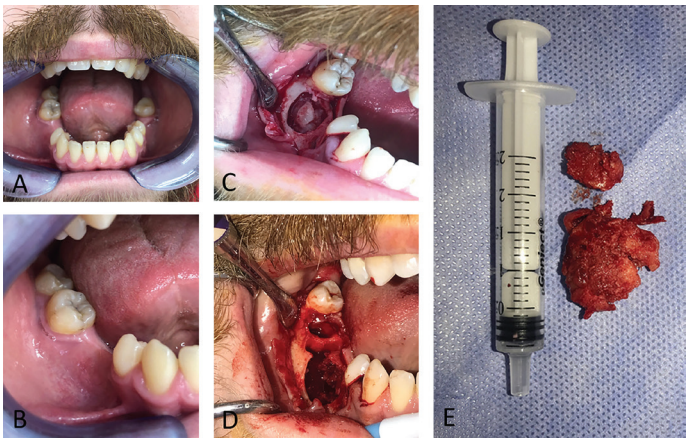
Case presentation

A 23-year-old male attending a private dental clinic for a routine dental check-up was referred to the oral surgery department for an abnormal radiolucency in the right mandible. He did not describe any of the five cardinal signs of inflammation, such as rubor (redness), calor (heat), tumor (swelling), dolor (pain) and functio laesa (loss of function). There was a history of a prolonged and painful healing period following the extraction of tooth no. 46

dating back to 4 years ago. He had no drug allergies or systemic disease, and denied any history of bad habits such as smoking tobacco, vaping or heavy alcohol consuming.

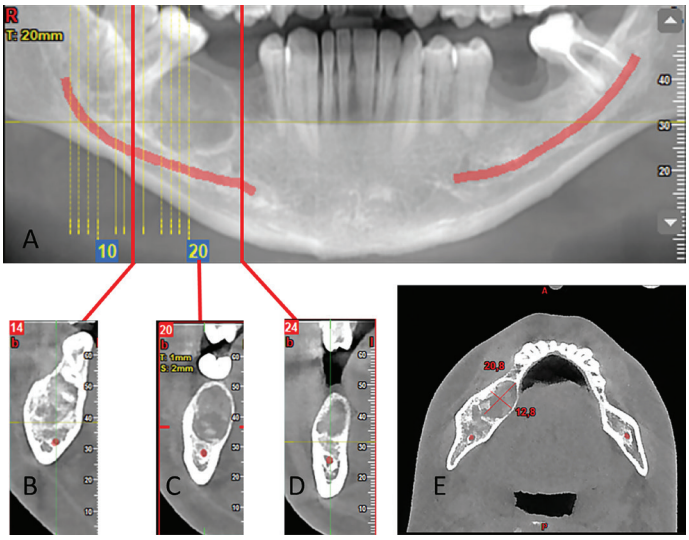
Extra-oral examination did not reveal either a firm or a mobile mass in the right lower jaw; however, intra-oral clinical examination revealed firm, non- tender moderate swelling around the alveolar ridge and on the buccal side of the right posterior mandible (Figure 1A-1B). On palpation, the swelling was firm in consistency. There was no pus discharge and bleeding on

Figure 1 - Intraoral photograph before operation (A, B). Preparation of bone window (C). After removing the mass (D). Specimen (E).



provocation. In the mandible, the swelling was extending from distal side of the second premolar to the second molar tooth region obliterating the buccal and the alveolar ridge corresponding to the region of extracted first molar tooth. There was also no complaint of facial or labial numbness. Orthopantomogram also revealed a lesion well-defined, expansive radiolucent mass with a number of scattered radiopaque calcified spots extending from the region of the previously extracted first molar to the roots of the second molar with a diameter of approximately 2×3 cm (Figure 2).

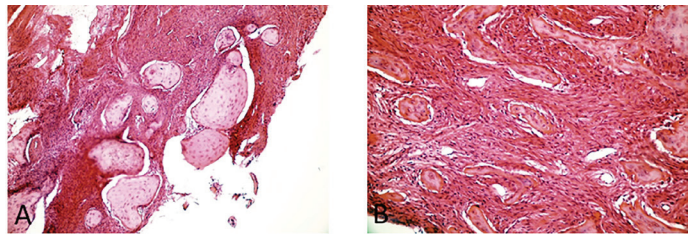
Figure 2 - Panoramic view (A). Cross-Sections on CBCT scans of the tumor (B-D). Axial CT image (E).



After the clinical examination, the mandible was scanned using a cone-beam computed tomography (NewTom Vgi evo, CeflaGroup, Verona, Italy) for further evaluation. Multiplanar images (Figure 2) provided detailed information on relevant roots of the second molar, cortical expansion and internal calcification. Examination of proximity to vital anatomical structures was also made prior of surgical intervention.

After informing the patient about the pertinent clinical findings, upcoming treatment and potential diagnosis of the lesion, the patient provided consent for the necessary operation. He underwent surgery under local anesthesia in the outpatient clinic. The regional nerve block was initiated with an inferior alveolar nerve (IAN) block using 2 ml of 40 mg/ml articaine with 12 mcg epinephrine. Subsequent buccal nerve block was also applied. A mucoperiosteal flap was raised from the region of the second premolar to the retromolar area. After flap elevation, the surgeon set about to preparation of a window by cutting into the thinned bone region seen over the translucency. The lesion was then completely removed in two pieces and the bony walls around the pertinent region curetted until sound tissue was macroscopically visible (Figure 1C-1D). The smaller specimen was labelled as "a small bony-like piece within the center" and the larger one was labelled as "other fully part of the lesion". The dimensions of smaller and larger specimens were 1×0.7×0.4 cm and 2.5×2.5×1.7 cm in aggregate, respectively (Figure 1E).

Figure 3 - Semento-osseous nature surrounded by lymphoplasmacytic infiltration (H&EX100) (A). Small and larger concentric cement foci and new bone trabeculae in a fibroblastic tissue (H&EX200) (B).



Microscopically (Figure 3), the decalcified smaller specimen demonstrated compact bone tissue and bone marrow fibrosis with angiogenesis in between. Microscopy of the larger specimen indicated that the lesion was characterized by spindle cell proliferation arranged in bundles and swirls. There were various sizes of calcifications most of which were in the form of bone trabeculae anastomosing with each other while some of which were resembles oval to round cementum-like structures. Based on all these observations, the lesion was diagnosed as COF.

The patient was prescribed amoxicillin/clavulanic acid 500 mg/125 mg po bid for a week and ibuprofen 600 mg po bid for 5 days. No adjuvant therapy was given. The patient had the checkup appointments at every week just for a month, and then he was informed about upcoming appointments arranged every 6 month for the next 2 years. Recurrence was not observed after one-year follow-up period.

Discussion

FOLs are generally defined as an uncommon condition in which normal bone tissue is replaced by a fibrous tissue containing a newly formed, mineralized substance. Although the definition of FOLs is fairly well established, there seems to be disagreement in the literature on definition of the different subtypes of FOLs. Charles Waldron proposed the first classification of FOLs of the jaws in 1985 [6]. In the Waldron classification, "ossifying and cementifying fibromas" were among "fibro-osseous (cemental) lesions presumably arising in the periodontal ligament" [7]. From a historical standpoint, while there were many amounts of proposals in classification, the WHO shed light on the current nomenclature and terminology of FOLs with their classifications in 1992, 2005, and 2017.

In 1992, the WHO grouped FOLs under osteogenic neoplasms and non-neoplastic bone lesions in the jaw. In this classification, both cementifying fibroma and ossifying fibroma lesions were listed under COF, as they overlapped with different histological variants of the same type of lesion [8, 9].

In the 2005 classification of odontogenic neoplasms, the WHO replaced the term "COF" with ossifying fibroma on the grounds of observation of cementum-like material of odontogenic origin in fibromas in extragnathic cases. It was clear that cementum and bone were actually the same tissue, to be differentiated according to their association with the tooth roots [8, 9].

Advances in classification have continued. The recent WHO (2017) edition of the classification of odontogenic and maxillofacial bone tumors introduced, for the first time, a lesion group of "fibro-osseous and osteochondromatous lesions". This edition re-added the prefix "cemento-" reintroducing cemento-ossifying fibroma (COF) as both a benign mesenchymal odontogenic tumor and a fibroosseous lesion (FOL) [1]. COF was therefore relocated into the benign mesenchymal odontogenic tumors category. It was also defined as a distinct type of ossifying fibroma that occurs in the tooth-bearing areas of the jaws and is believed to be of odontogenic origin [1, 5]. Nevertheless, the acronym COF was used throughout the relevant section on ossifying fibroma except for juvenile ossifying fibroma (JOF) variants: juvenile trabecular ossifying fibroma and juvenile psammomatoid ossifying fibroma [1]. Therefore, there is a proposal to reconsider conventional ossifying fibroma as comprising odontogenic and non-odontogenic subtypes because of several extragnathic cases reported [5].

The origins of COF and the other recognized FOLs are actually complex and not well understood. Most cases reported in the literature were associated with a previous trauma history [9, 10]. Another concept related to its origin is based on developmental causes. A few reports have pointed out extragnatic cases originating from embryologic nests [11, 12]. In the present case, the patient had a history of delayed healing, with concominant purulent discharge, following the extraction of tooth no 46. Considering that there were no previous radiological records either electronic or in hard copy format, we could not be sure whether the presence of the lesion was overlooked by the clinician. From a clinical standpoint, if there is prolonged or unusual healing following dental treatment, suspicion for any kind of lesions is crucial as early diagnosis is important for an effective cure [13]. Clinicians should consider that delayed diagnosis of any kind of lesion can result in more advanced disease at the time of treatment, potentially leading to the patient's frustration and loss of confidence in the healtcare system, greater cost and higher morbidity [13, 14].

The COF is one of the most recognized FOLs along with to FD and COD. Although histologically similar, they can be distinguished by their clinical and radiological features. Clinically, a COF shows a slow- growing pattern, and evolves in young and middle-aged adults with a predilection for women. Traditionally, COF has an ovoid shape owing to a centrifugal growing pattern resulting in growth from the center to the periphery and presenting as a round tumor mass. Looking more closely at demographic factors, COF is prone to occur in the second to fourth decades of life, with a noticeably higher proportion among women, at a ratio of 5:1 [9, 15]. In addition, a COF may occur in any tooth-bearing areas of the mandible and maxilla, most commonly in the posterior side of mandible. In our case, while clinical presentation matched location predilection, there is a contradiction about the sex predilection [16].

Radiographically, a COF appears as a generally well-demarcated and unilocular lesion, which displays radiolucency with variable radiopaque foci depending on the amount of mineralization. In a retrospective study conducted by Titinchi et al. [17], it was reported that approximately 15.9% of lesions would be radiolucent and multilocular. Nevertheless, if there is an impacted tooth, it is difficult to distinguish a COF from either a calcifying epithelial tumor or a calcifying odontogenic cyst based on its radiographic appearance [9, 18]. Moreover, the differential diagnosis of COF involves not only both a calcifying epithelial tumor and a calcifying odontogenic cyst, but also ameloblastic fibroodontoma, cementoblastoma, odontoma, and fibrous dysplasia [9]. In the present case, the radiologist made a provisional diagnosis of ameloblastic fibroodontoma or cemento-ossifying fibroma. On the radiographic examination, displacement of teeth and root resorption may be seen in COF [6, 9]; however, in the present case, radiographic and CT scan images did not reveal any displacement or resorption related to the neighboring tooth. Thus, our diagnosis was made on the basis of the histopathological report.

Microscopically, a COF represents a hypercellular fibroblastic stroma with many delicate bundles of spindle shaped collagen fibers, proliferating fibroblasts, cementoblasts and variable amounts of calcified structures [6]. The latter consist of osteoid bone and hypocellular basophilic structures of cementum-like tissue resembling the cementicles that are normally seen in the periodontal membrane [19].

The mainstream treatment of COF depends on its clinical and radiological features and includes one of the following methods: enucleation, curettage, or surgical resection. While enucleation and primary closure can be performed for smaller lesions, moderate lesions require a more cautious approach with local excision and curettage up to a point at which sound tissue becomes macroscopically visible. A larger COF will need a bone resection as a radical approach to guard against the high tendency to relapse following incomplete removal [17]. In such cases, the resection has to be performed in alignment with bone reconstruction, either by an iliac crest nonvascularized bone graft or a free fibula flap. The most common complications in such operations are flap dehiscence and graft exposure, which may be treated with a second surgery at a time when soft tissue maturation occurs [17-20]. In the present case, the patient was treated thoroughly with local excision/curettage of the lesion regarding a little cleavage margin on radiographic examination.

Conclusion

In conclusion, distinguishing between COF and other lesions and benign tumors is a challenge and a decisive factor in the proper diagnosis. On this basis, oral surgeons should be aware of different aspects of radiographic findings and should regard the pathologists' report due to the definitive diagnosis being mostly based on them. Besides prompt evaluation, accurate diagnosis and proper treatment of the lesions, long-term follow-up is crucial to evaluate recurrence for all patients, regardless of the type of surgical management.

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A clinical case of an immunosuppressive generalized form of Kaposi's sarcoma in a patient with pemphigus vulgaris

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Abstract

The article presents the literature data on Kaposi's sarcoma a lymphangioproliferative neoplasia induced by the Herpes Virus type 8. The main forms, clinical manifestations and treatment are described. A clinical case of the development of an immunosuppressive generalized form of Kaposi's sarcoma induced by glucocorticosteroid therapy in a patient with pemphigus vulgaris is presented. With this clinical example, it is important to emphasize the potential risk of Kaposi's sarcoma on the background of secondary immunosuppression. Immunosuppressive Kaposi's sarcoma (iatrogenic type) is most often associated with long-term use of immunosuppressive therapy in transplantation organs and in patients receiving immunosuppressive therapy for autoimmune diseases, which leads to an increased risk of developing Kaposi's sarcoma by 150-1000 times compared with the general population. The ratio of men and women with this type is 2:1, while with the idiopathic (classical) - 17:1. Reliable diagnosis of the disease is necessary, based on a combination of history data, clinical and histological patterns of the pathological process, as well as additional laboratory markers, which will allow timely determination of further patient management tactics and, accordingly, provide a more favorable prognosis for the course of the disease.

Key words: Kaposi's sarcoma, HHV type 8, clinical case, secondary immunosuppression

Introduction

Kaposi's sarcoma (KS) is a systemic multifocal tumor of endothelial origin with a primary lesion of the skin, lymph nodes, and internal organs [1]. KS was first reported in 1872 by the Austro-Hungarian dermatologist Moritz Kaposi under the name "idiopathic multiple pigmented sarcoma" in elderly European men with multiple cutaneous and extracutaneous neoplasms, all of whom died within 2 years. In 1898, G. Koebner proposed the term "Kaposi's sarcoma" after the name of the discoverer of the disease [2]. The development of Kaposi's sarcoma is most likely in individuals belonging to the following risk groups: men of Mediterranean origin over 60 years old, a number of Central African countries, HIV-infected men, transplant recipients or patients receiving long-term immunosuppressive therapy [3]. Men get sick 9-15 times more often than women.

Currently, 4 variants of KS are distinguished, which have peculiar epidemiological characteristics and clinical course, but have comparable histopathological features [4,5]:

1. Classical (idiopathic, sporadic) KS is common among elderly men of Mediterranean or Jewish descent and, unlike the cases originally described by Kaposi, usually has a sluggish, protracted clinical course and

primarily affects the skin on the legs. As a rule, it begins as a localized reactive inflammatory-angiogenic process of the skin and can slowly progress to a real sarcoma, in rare cases with damage to internal organs [5].

2. Endemic (African) SK - common in some countries of Central Africa. There is a chronic type, which does not differ from the classical form, and a rapidly progressing lymph adenoid-like variant, characteristic of childhood, with a fatal outcome 2–3 months after the onset of the disease [6].

3. Immunosuppressive (iatrogenic) SK, which develops under the influence of immunosuppressive therapy of various origins, most often occurs after transplantation of various organs [7].

4. Epidemic (AIDS-associated) SK - rapidly progressing in HIV-positive patients, characterized by the early formation of extracutaneous lesions.

According to the literature, there is currently no consensus on the pathogenesis of KS. The origin of spindle cells from the endothelium of lymphatic vessels is considered [8]. However, the lymphatic or vascular nature of these cells is still a matter of debate. Indeed, spindle cells express both vascular and lymphatic endothelial cell markers (VEGF-3, LYVE-1 and podoplanin or CD34, CD31 and CD36, respectively) and have the phenotypic characteristics of two cells. The cause of KS was not known until 1994, on the basis of epidemiological assumptions an infectious origin independent of HIV was proposed, a directed search led to the discovery of human herpes virus type 8 (HHV-8). The HHV-8 genome is found in all elements of the SC in a latent form, at all stages of the disease, regardless of the clinical variant, however, a small proportion of viral particles (<5%) is in a replicative state and, as reported, is potentially involved in the proliferation of neighboring cells, which suggests that they play a crucial role in the process of oncogenesis [9]. The immune response to HHV-8 paradoxically exacerbates the reactive process, contributing to the transition to true sarcoma. In the classical form of KS, there is no change in the specific acquired immunodeficiency in both T-cell populations, while in the non-classical form of KS, initiation and progression are modulated in the immune system [10].

The predominant localization of foci of rashes is the lateral surfaces of the legs, feet, and hands. The lesions are usually symmetrical, with clear boundaries. Subjectively, an asymptomatic course is more often characteristic, sometimes itching and burning are possible. There are 3 clinical stages of KS:

1. Spotted - reddish-cyanotic or reddish-brown spots, 1-5 mm in diameter, irregular in shape, with a smooth surface, asymmetrically or symmetrically arranged [11,12]. Considered as an early stage, characterized by slow horizontal and vertical growth, with a tendency to progress to hard plaques and occasionally to nodules.

2. At the papular stage - the presence of papules of a spherical or hemispherical shape, densely elastic consistency, 0.2-1 cm in diameter is noted. Papules tend to group into plaques with a smooth or rough orange-peel surface, sometimes with papillomatous growths [13].

3. Tumor stage - characterized by the formation of single or multiple nodes up to 1-5 cm in diameter, soft or densely elastic consistency, with a tendency to merge and ulcerate [14]. Edema often occurs around the tumor, accompanied by the formation of depressions when pressed. Such edema can transform into fibrosis. The color of the lesions changes to brownish, hyperkeratosis of the proper skin develops, and ulceration develops, especially on the lower extremities. Ulcers are deep, irregularly shaped, with twisted edges, bluish in color and tuberous, bloody-gangrenous bottom, sharply painful. After several years of progression, the spread of KS to other parts of the body is often detected and tumors can be found on the mucous membranes of various organs, including the oral cavity and/or genitals. There are also lesions of the internal organs, respiratory organs, lymph nodes [9,14], where they rarely manifest any symptoms [3].

The course of KS can be acute, subacute and chronic. Life expectancy is from 2 months up to 2 years [4].

The histopathological picture depends on the stage of KS development. Early patchy elements are characterized by an increase in the number of vessels in the surface layer of the dermis, surrounded by irregularly shaped endothelial cells. Vessels run parallel to the surface of the skin, are often tortuous and can form bizarre cracks and fissures. In the adjacent skin, areas of hemosiderin deposition and extravasal erythrocytes, as well as a moderate inflammatory infiltrate, are often detected. In the plaque stage of KS, marked vascular proliferation is noted in all layers of the dermis with multiple dilated and angular vessels that traverse collagen. The nodular foci of KSs consist mainly of spindle-shaped cells arranged in the form of fibers and alternating bundles with disordered uneven slit-like vascular zones without endothelial lining. More developed elements may show pronounced pleomorphism, nuclear atypia, and mitotic figures. Along the periphery of solid tumors, lymphangiomatous areas with bizarre vascular lumens, intra and extravascular erythrocytes, and siderophages can be found. A moderate inflammatory infiltrate consisting of lymphocytes, histiocytes, plasma cells, and, rarely, neutrophils is almost always detected at all stages of KS [3].

The main goals of treatment are to prevent disease progression, reduce swelling and, prevent organ damage, and relieve psychological stress. Therapy for KS should be selected depending on the subtype, stage of the disease, and taking into account the immune status of the patient [15]. With regard to the herpes virus, there is no eradication treatment for HHV-8. This fact makes scientists doubt the possibility of curing any form of KS. Targets in the treatment of KS are also HIV (in patients with an AIDS-associated form of the tumor), the processes of angiogenesis and cell differentiation. Local methods of treatment include: surgical treatment, liquid nitrogen destruction, topical therapy using 9-cis-retinoic acid, imiquimod [16], interferon, local chemotherapy [3]. The method of photodynamic therapy (PDT) [17] is also used, which is minimally invasive, has a high selective activity of tumor lesions, low toxicity, and no risk of severe local and systemic complications. The most commonly used prospidin, which is characterized by high tropism to the skin and no ambivalent effect after withdrawal, hemotoxic effect [8,18]. Depending on the immune status of the patient, interferon 2, carbamoylaziridine, IFN-α, IL-12, etc. are additionally used during prospidin therapy [18]. It is assumed that they cause apoptosis of SC cells [19]. In patients with rapidly progressive or advanced classical KS, especially with involvement of internal organs, chemotherapeutic agents are widely used as monotherapy or combined treatment [3].

Case presentation

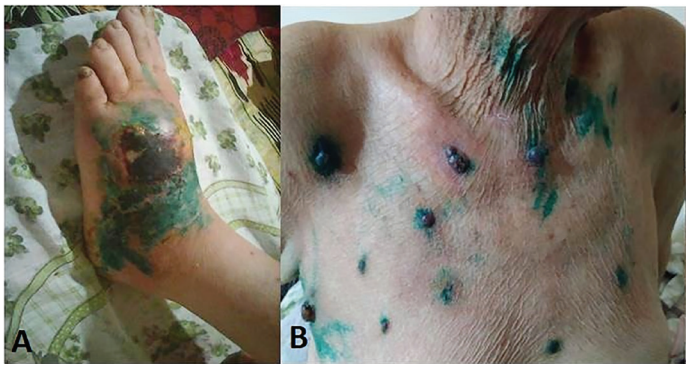
We present our clinical observation of the development of an immunosuppressive generalized form of Kaposi's sarcoma in a patient with pemphigus vulgaris. Patient S., 73 years old, who received regular inpatient treatment for pemphigus vulgaris for 6 years, was discharged after another hospitalization on 11 tablets

(55 mg/day) of prednisolone with gradual dose titration. With a decrease in the dose of prednisolone to 20 mg/day, he noted the appearance of rashes on the left foot, swelling and soreness. He was examined by a surgeon at the place of residence, where an incision was made with suspicion of phlegmon. From the words, no signs of phlegmonous inflammation were detected. After 1-week, purple spots began to appear on both legs, gradually increasing in size. After 1-month, similar rashes appeared on the body, soreness, weakness, inability to move independently, in connection with which the patient turned to the Republican Dermatovenerological Clinical Hospital of the Ministry of Health of the Republic of Uzbekistan.

Status preasens: General condition of moderate severity. The patient is lying down, does not move independently. Normosthenic body type. On auscultation in the lungs, hard breathing, single wheezing is heard. The borders of the heart are deviated to the left. Stool - there is a tendency to constipation.

Status localis: The skin pathological process is widespread, symmetrical, chronic inflammatory. Localized on the skin of the lower and upper extremities, the anterior and posterior surface of the body. The elements of the lesion are represented by papules, plaques, nodes from 1 to 5 cm in diameter, bluish-red color, hemispherical shape with sharp borders, rounded outlines, in some places covered with a small amount of scales. Their surface is uneven, bumpy. The lower extremities are edematous, the skin is infiltrated. On the feet there are nodes of a bluish-purple hue, with an ulcerated surface, multiple erosions, ulcers with purulent discharge. There is a specific putrid smell. Visible mucous membranes are free from rashes. Subjectively, the patient is concerned about moderate pain in the area of the rash. Rashes that speak for pemphigus in the patient were not observed (Figure 1).

Figure 1 - A) Patient C. Nodules of a bluish-purple color with an ulcerated surface, multiple erosions, ulcers with purulent discharge. B) On the skin of the body - papules, plaques, nodes of bluish-red color from 1 to 5 cm in diameter, hemispherical in shape with sharp boundaries

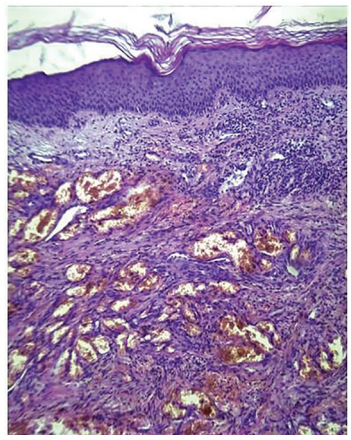


According to clinical and laboratory data: Hypochromic anemia was detected in the KLA; In OAM - traces of protein, salts of uric acid. Biochemical analysis of blood - no pathology. HIV test is negative. In the immunogram, there is a decrease in the relative and absolute values of the total pool of T-lymphocytes, an imbalance in the immunoregulatory populations of T-lymphocytes. IRI is suppressed. Increased the number of natural killers. SD level 4 - 315 (N-580-1110).

To clarify the clinical diagnosis, with the consent of the patient, a diagnostic skin biopsy was performed from the lesion in the shin area.

Histological examination of the skin: A slight hyperkeratosis was revealed, in some places detachment of the stratum corneum. Slight acanthosis, granular layer without features. In the dermis, chaotic proliferates of slit-like and wide-lumen capillaries are noted, represented by atypical thin-walled vessels. In the middle third of the dermis, there is an accumulation of spindle-shaped cells, hemosiderin deposition, collagen fibers are fibrously changed in places. Skin appendages are not defined (Figure 2). Conclusion: Kaposi's sarcoma, angiomatous type.

Figure 2 - Histological picture of a biopsy specimen from the skin of the body, stained with Hematoxylin-eosin. Zoom x 100



Based on complaints, anamnesis (long-term use of corticosteroids), the result of a biopsy, the diagnosis was made: Kaposi's sarcoma, a generalized form of the immunosuppressive type. After making the final diagnosis and consulting an oncologist, he was transferred to the Republican Oncology Center for further treatment. However, 5 months later, against the background of the progression of the process, the patient died.

Conclusion

Thus, taking into account the literature data, the immunosuppressive type of SC can occur with prolonged use of GCS, which is not excluded in our patient, and the trigger for the rapid progression of the process may have been surgical manipulation, after which, according to the patient, bluish spots began to appear literally every day on the legs, and then gradually all over the body. Interest in the study of MC, despite the fact that it was described more than 140 years ago, is associated with an increase in the incidence of this disease, including against the background of HIV infection, immunosuppressive therapy for chronic systemic diseases, organ and tissue transplantation. However, the lack of efficiency in diagnosing and treating various types and forms of KS, the lack of consideration of the stage of the disease, the prevalence of the pathological process, the degree of immunosuppression, the severity of side effects from the therapy, makes this disease a serious interdisciplinary problem at the moment, which is faced not only by dermatologists, oncologists, rheumatologists, but also doctors of other specialties. With an integrated approach to the diagnosis and treatment of SC, it is possible to effectively control the tumor process, which makes it possible to achieve a sufficiently long remission, and in some cases, a complete regression of the pathological process.

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Surgical correction and postoperative period management of a patient with the giant left atrium: case report

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Abstract

This case report describes the history of a 62-year-old man with symptoms of severe heart failure caused by critical mitral valve (MV) stenosis with extremely enlarged left atrium (LA) and chronic rheumatic heart disease. The patient has had MV replacement, aortic valve (AoV) replacement, tricuspid valve (TV) plastic surgery, left atrial thrombectomy, reduction, and resection of the LA.

The operation was performed by median sternotomy, in condition of artificial blood circulation with aortic cannulation, separate cannulation of Cava veins and hypothermia up to 29 C. Selective antegrade cold blood cardioplegia was used for the myocardium protection.

Revision of the MV was performed via the right atrium. Findings: the valves deformed by severe fibrosis, thickened subvalvular structures. Also, there was a massive thrombosis of the LA cavity. A thrombectomy was performed, total amount of evacuated thrombotic masses - 350 ml. Considering the circumstances of atriomegalia, was decided to provide reduction of the LA. MV replacement with mechanical prosthesis SJM MastersSeries # 31, aortic valve replacement with prosthesis SJM MastersSeries #25, and Suture commissuroannuloplasty were performed.

We believe that our surgical tactics has effectively reduced the size of the LA, together with the correction of valvular malformation, can significantly improve the patient's life quality.

Key words: mitral valve replacement, aortic valve replacement, thrombectomy, valvular malformation, resection of the left atrium

Introduction

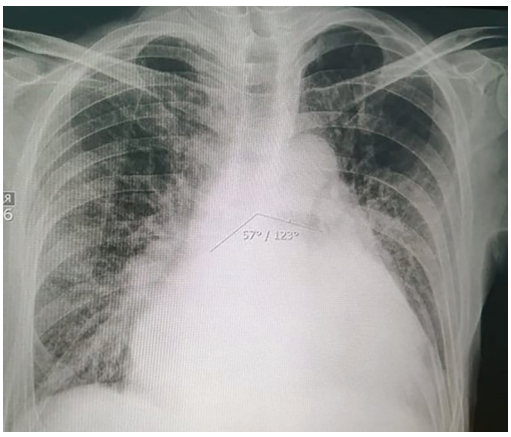
Surgery of mitral valve defects is still an urgent problem of modern cardiac surgery [1-3]. Changes in the mitral valve because of the rheumatic process, infectious endocarditis, leads to the development of mitral malformation as a result, impairment of intracardiac hemodynamics. Narrowing of the left atrioventricular opening makes difficulties to blood passing from the left atrium to the left ventricle, causing tonogenic overload of the left atrium. Pathological changes subsequently lead to an increase sizes atrial cavity. Surgical treatment of mitral valve defects with giant left atrium is associated with a high mortality rate from 8 to 32% [4]. In patients operated for atriomegaly according to summary data is variable from 7.8 to 19 % [5,6]. Atriomegaly can lead to compression of Vena cava, right chambers of the heart, reduction of venous return, compression wall of the left ventricle, and also cause compression of extracardiac organs and tissues – such as bronchial tree (an increasement of the Carina angle more than 120 degrees), basal segments of the lung's, esophagus, descending part of the aorta with corresponding symptoms [7-9]. In this regard, in case of extreme atriomegaly with mitral valve defects, atrial reduction should be considered as an addition to valve surgery.

Case presentation

A 62-year-old man was admitted to the National research cardiac surgery center with symptoms of severe heart failure caused by critical mitral valve stenosis

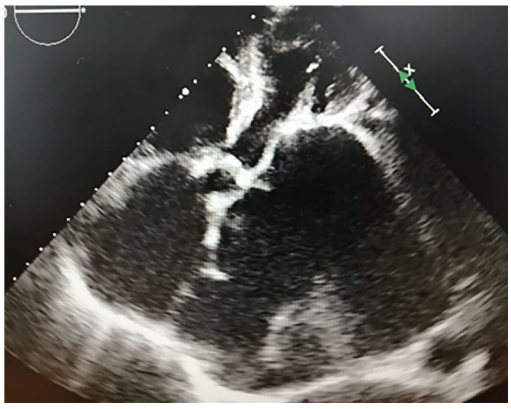
with extremely enlarged left atrium (LA) and chronic rheumatic heart disease. Past history: more than 40 years ago diagnosed rheumatic disease, atrial fibrillation (date is unknown), officially registered more than 4 years ago. On the X-ray examination picture of severe dilatation of the left atrium, a shift of the Carina angle to 123 degrees (Figure 1).

Figure 1 - Chest X - ray. Shift of the Carina angle to 123 degrees



The aorta and the trunk of the pulmonary artery were cut off transversely in order to mobilization of the LA. The dilated LA wall was excised while preserving the physiological anatomy

Figure 2 - Echo. Perioperative period.



According to echocardiography (EchoCG) data at the moment of surgery, the size of the LA was 9.8cm x 7.3 cm, the volume of the LA about 600 ml, and the area was 96 cm2 (Figure 2).

Patient also had a moderate degree of aortic and mitral valve insufficiency. Signs of thrombosis in the left atrial cavity were detected. According to EchoCG data the pulmonary artery systolic pressure (PASP) was 70 mmHg. Coronary angiography had shown intact coronary artery.

Surgical treatment was performed to patient. The operation was performed by median sternotomy, in condition of cardiopulmonary bypass (CPB) with aortic cannulation, separate cannulation of Cava veins and hypothermia up to 29 C. Selective antegrade cold blood cardioplegia was used for the myocardium protection.

Revision of the mitral valve was performed via the right atrium and interatrial septum access. Findings: the valves deformed by severe fibrosis, thickened subvalvular structures. Also, there was a massive thrombosis of the LA cavity. A thrombectomy was performed, total amount of evacuated thrombotic masses - 350 ml. Considering the circumstances of atriomegalia, was decided to provide reduction of the LA. of the atrium in relation to the heart structures, followed by atrioplasty and reduction of the left atrium cavity. Enlarged wall of the left atrium was resected in a band 2-3 cm wide from the upper border of the LA, laterally, then to the posterior wall, the incision was sutured along the section line. Mitral valve replacement with mechanical prosthesis SJM MastersSeries # 31, aortic valve replacement with prosthesis SJM MastersSeries #25, and Suture commissuroannuloplasty were performed. In the early postoperative period bleeding (total amount of blood loss about1600 ml) caused by coagulopathy and initially impaired liver function, due to active viral hepatitis C.

Management in postoperative period in cardiac intensive care unit (CICU).

The patient was delivered to the CICU from the operating theatre at 3:05 p.m. on 18.10.2020. The patient has had mitral valve replacement, aortic valve replacement, tricuspid valve plastic surgery, left atrial thrombectomy, reduction, and resection of the LA 18.10.20. The duration of cardiopulmonary bypass was 279 minutes.

Clump cross time - 224 minutes. Circulatory arrest time - 1 minute.

Respiratory therapy: The duration of artificial lung ventilation 23 hours 55 minutes. Tracheal extubation was performed on 19.10.2020 2:40 p.m. From 19.10.2020 to 20.10.2020 non-invasive ventilation (NIV) was performed in the CPAP mode. NIV without positive effect. During NIV there was limitation of mobility of the patient, difficulties with enteral feeding. Respiratory therapy changed to high-flow nasal oxygen therapy (HFNOT) on 21.10.2020, with parameters: flow 50 l/min, FiO2-50%. Activation of the patient (vertical position, sitting in the chair, walking around the ward) was started at time of HFNOT.

Figure 3 - Chest X-Ray in dynamics (24 hours after surgery)

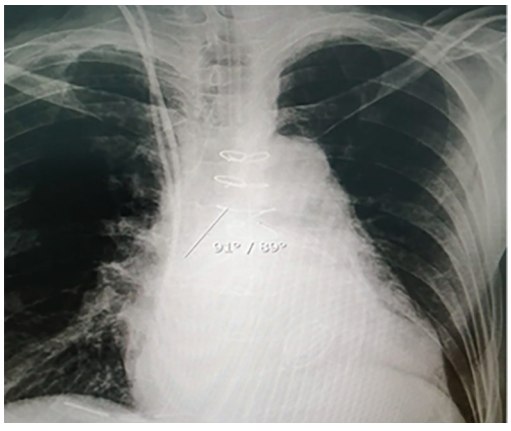
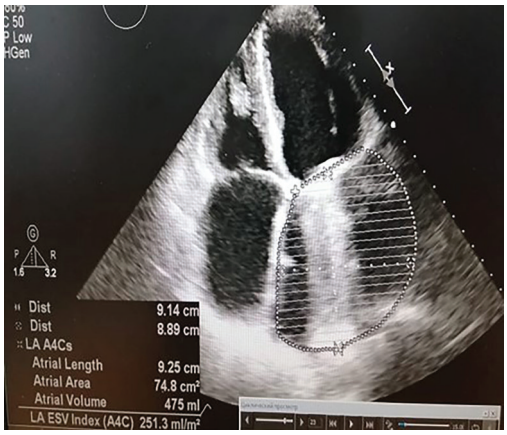


Figure 4 - Echo (24 hours after surgery).



In dynamics, the chest X-ray had shown a decrease of the Carina angle to 89 degrees (Figure 3).

EchoCG confirmed a reduction of the size of the LA: the volume of the LA 475 ml, the surface area 74.8 cm2 (Figure 4).

During postoperative period in ICU, severe prolonged respiratory failure. Dynamics of respiratory status represented on the Table 1.

Table 1 Respiratory status

	PaO2/FiO2	pCO2	pH
Before operation	393	37,8	7,42
After operation	302	43,1	7,35
Before extubation	572	50,5	7,43
After extubation	532	48,8	7,34
CPAP	470	52,9	7,34
CPAP	400	57,8	7,43
CPAP	276	64,2	7,39
HighFlow	276	58,6	7,44
HighFlow	440	50,5	7,49
		47	7,36

Discussion

A significant enlargement of LA has a poor prognostic effect for patients who are undergoing valve repair or replacement with a prosthesis due to mitral valve damage [10]. Measurement of the left atrium on two-dimensional EchoCG is one of the most important factors determining the outcome after mitral valve replacement. There are several distinctive pathophysiological changes associated with giant LA [11]. One of them is respiratory diseases due to compression of the left main bronchus and / or the right middle and lower lobes of lung by dilated LA [12].

We believe what the cause of respiratory failure in our case was regarded as the expansion of long-term atelectasis of the lower lobe of the left lung caused by compression of the giant left atrium. In the preoperative period, compensation for the condition was caused by a decreasing in ventilation of the lung section with atelectasis. Also, was parallel decreasing in its perfusion, which did not cause gas exchange disorders. In the postoperative period, after the reduction of the LA, the lower lobe of the left lung was released from compression, as a result, atelectasis was straightened with the restoration of ventilation and perfusion functions. Subsequently, in this area was started intensive production of sputum, this led to a impairment of the ventilation of the lung section, with preserved perfusion, in consequence was formed pulmonary venous-arterial bypass (mixing of venous blood with arterial blood, which increases the degree of hypoxemia and hypercapnia).

Another is an infringement of hemodynamics due to compression of the posterior basal part of the LV due to the expansion of the LA downwards [13].

In addition, the presence of significantly increased blood pressure (BP) may increase the risk of thromboembolism, regardless of antithrombotic and antiaggregant therapy [14,15]. Volume reduction can be achieved by forming a fold in the LA wall, but resection can do more extensive volume reduction.

We believe that our surgical tactics has effectively reduced the size of the LA, together with the correction of valvular malformation, can significantly improve the patient's life quality. We believe that surgical correction of the dilated LA is compulsory in patients with mitral valve damage and giant LA, especially in the presence of atrial thrombosis, displacement of the left bronchus and impaired respiratory function. The postoperative period is not standard. It has many unforeseen complications. In early postoperative period in CICU department intensivist should be prepared for a long-time collision with respiratory failure, which was absent or slightly presented before surgical operation. Intensivists must detect symptoms of complications on the early stage and have a way to solve them.

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COVID-19 associated rhino-orbital-cerebral mucormycosis with underlying diabetes mellitus

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Abstract

Introduction: Since the beginning of the COVID-19 pandemic, there has been an increase in opportunistic infections such as mucormycosis, which is of some concern given the fairly rapid spread. The development of immunosuppression associated with COVID-19, coupled with the presence of comorbidities that exacerbate the progression of the disease and in some cases lead to a fatal outcome, plays an important role in this.

Case presentation: We have examined a real case of rhino-orbital-cerebral mucormycosis in comparison with earlier published contemporary studies on epidemiology, clinical manifestations, and risk factors. Autopsy materials of nasal mucosa, eye socket, brain of a patient with COVID-19 associated with diabetes mellitus have been subjected to a histological study.

Conclusion: The presented case demonstrates a unique pathomorphological pattern of rhino-orbital-cerebral mucormycosis, first registered in Kazakhstan and of great interest in the world practice.

Key words: mucormycosis, diabetes mellitus, COVID-19, corticosteroids

Introduction

The global COVID-19 pandemic, which is widespread throughout the world, is a threat to public health, socio-economic livelihoods and humanity as a whole. Clinical manifestations of the disease can vary from asymptomatic to severe forms. Regardless of the age of the patients, the mortality rate can be affected by the presence of comorbidities of the cardiovascular system, liver and kidneys, as well as diabetes mellitus [1-2].

Severe forms of COVID-19 are accompanied by bacterial and fungal infections. One of these opportunistic infections is mucormycosis, an upsurge of which has been reported worldwide [3]. Thus, the disease incidence in India rose to 26.7%. A significant role in the development of invasive forms is played by a weakening of the immune system due to a severe course of diabetes mellitus, immunodeficiency, cancer and transplantation. In clinical practice, quite often there are lesions of the sinuses, brain, lungs and other regions in patients with COVID-19. In the rhinocerebral form of mucormycosis, the most typical location is in the nose, but the disease may spread to the paranasal sinuses, orbit, facial bones, and skull cavity. The administration of glucocorticosteroids in the treatment of COVID-19 reduces resistance to opportunistic infections. The prevalence of clinical and radiological symptoms of coronavirus pneumonia makes it difficult to diagnose mucormycosis. This leads to late diagnosis and unfavorable prognosis [4-6].

According to a systematic review of 101 cases of mucormycosis of different localizations in patients with confirmed COVID-19 by Indian scientists Singh A.K. et al., 82 cases were reported in India, while there were 19 cases in other countries [7]. Nasal and sinus lesions were the most common (88.9%), followed by rhino-orbital (56.7%) and rhino-orbital-cerebral forms (22.7%). The leading risk factors in these cases were the presence of diabetes mellitus and COVID-19 therapy with glucocorticosteroids. Intracranial or orbital lesions associated with the immunosuppression drive a high mortality rate of 50-80%. Tissue necrosis is a distinctive feature of mucormycosis, but it appears late [7-8].

Our study shows what pathomorphological changes have been found in rhino-orbit-cerebral mucormycosis lesions in a single fatal case in Kazakhstan. The case report will be an instructive, scientific and educational tool for the work of pathologists, forensic experts of the world for the reason that in most cases of cerebral mucormycosis lesions in COVID-19 autopsies have not been conducted to avoid spread of infection, while we in Kazakhstan have performed autopsies followed by macro and microscopic examination of tissues.

Case presentation

Medical experts from Kazakhstan found a single fatal case of mucormycosis in COVID-19 with rhino-orbital-cerebral lesions in a 16 year old patient. After autopsy, a histological examination was performed. From the brief fact of the case, we know that the patient had a history of diabetes mellitus in her life. During the pandemic, she contracted COVID-19 infection identified by PCR assay (polymerase chain reaction). On admission to the hospital, her health status was assessed as extremely severe. The patient was in the intensive care unit for several days. While in the intensive care unit, doctors noticed that the girl gradually developed black spots on her face, mostly in the area of her nose.

Despite the ongoing therapy (which included glucocorticosteroids), the patient developed coma and died. Provisional forensic diagnosis: Cerebral infarction (in question), COVID-19 associated pneumonia (in question), Nasal septal mucosa necrosis of unknown genesis.

Due to the disfigurement of the face with blackened skin color, the parents complained to the investigators about the actions of doctors and suspected oxygen burns of the face in the intensive care unit, so a forensic medical examination was appointed.

On autopsy, macroscopic examination showed: the internal organs were anatomically correctly positioned. Changes were found in the brain with predominant brown softening in the frontal lobe in the area of the olfactory nerve and basal part in the region of the oculomotor nerve on the left. In the lungs, in the pleural cavity, hemorrhagic fluid with overlapping fibrin strands, lung parenchyma of dense consistency, red in color on section. The pancreas was small and lobular in shape, of dense consistency.

Topically: necrotic changes of the soft tissue of the nasal mucosa, facial skin in the orbital region with bruising and black tint, mainly in the nasal region with necrotic destruction of the nasal septum, extending to the eye socket with scab and facial disfigurement. Figure 1 (A, B, C) shows the fungal lesion region with facial disfigurement.

Figure 1 - Fungal lesion region with facial disfigurement: A, Necrosis of the skin and subcutaneous tissue in the form of a black scab from the nasal cavity area. B, Necrosis of the skin of the bridge of the nose, zygomatic region and eyelids of the eye socket. C, Necrosis of skin and mucous membrane of eyelid and sclera in the form of black scab.

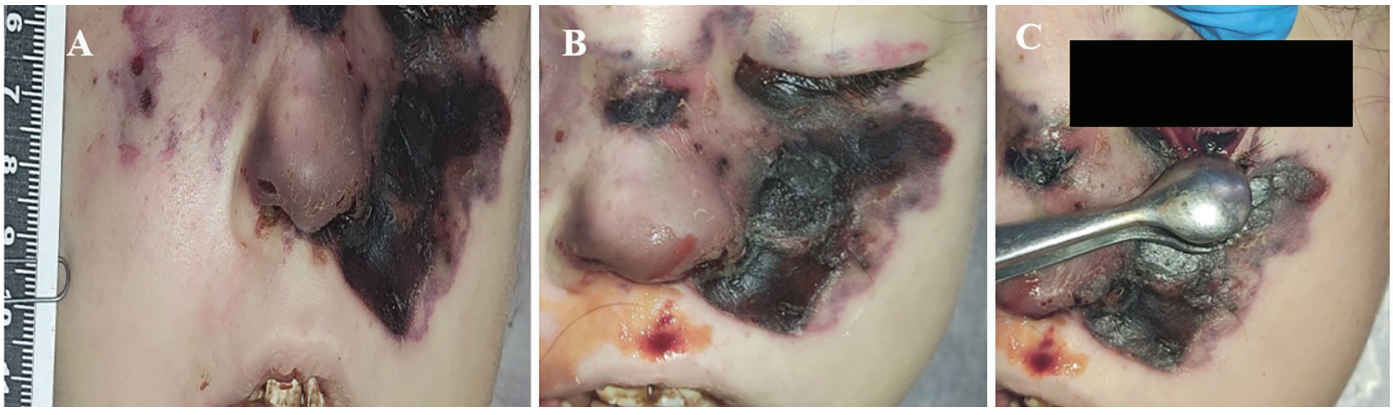


Figure 2 - Photomicrograph of a histological examination of necrotic nasal cavity tissue with a cluster of mucormycosis bacilli, fungal hyphae of mucormycosis. Hematoxylin and eosin, x 200.

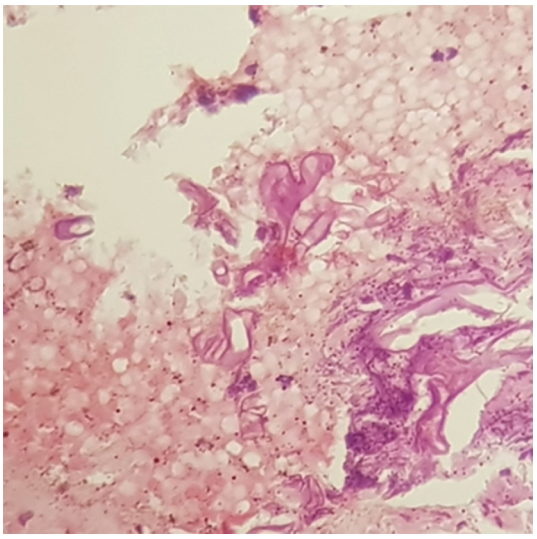


Figure 3a - White matter of the brain with glial reaction to introduction of mucormycosis hyphae, spherulas. A, branches of mucormycosis hyphae and spherules in the white matter of the brain. Hematoxylin and eosin staining, x150.

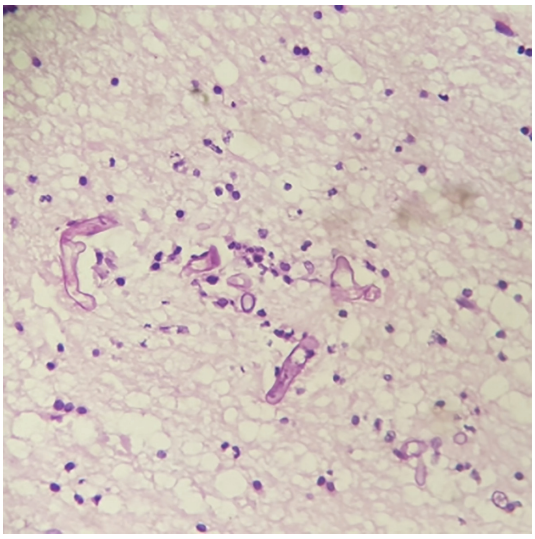


Figure 3b - B, Same area of white matter at low magnification with glial reaction to accumulation of mucormycosis hyphae and spherulas. Hematoxylin and eosin, x40.

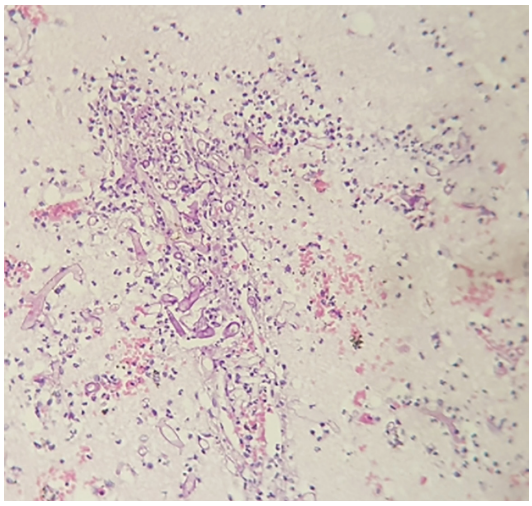


Figure 4a - Grey matter lesion of the cerebral cortex with mucormycosis. A, Massive accumulation of mucormycosis bacilli with glial-cellular reaction. Hematoxylin and eosin, x 400.

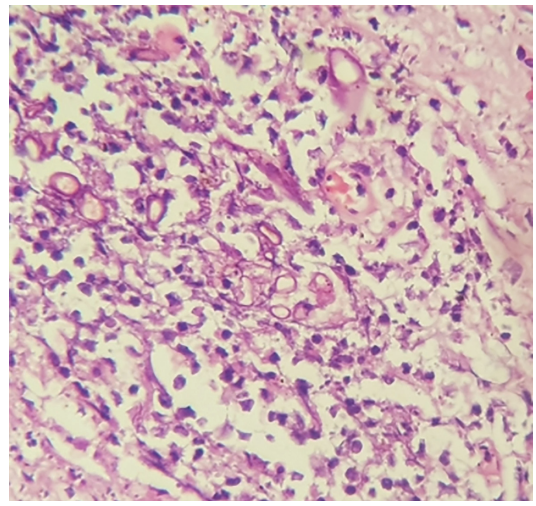


Figure 4b - B, Ring-shaped accumulation of mucormycosis hyphae and spherulas with perifocal cellular reaction and small foci of hemorrhage. Hematoxylin and eosin, x40.

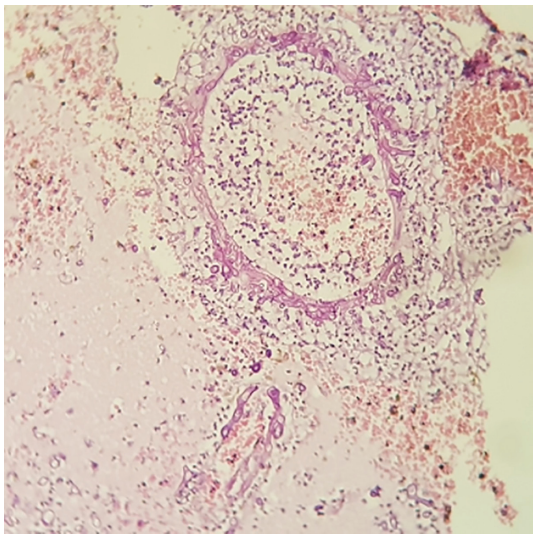


Figure 5a - Photomicrograph of the brain with mucormycosis lesions: bacilliform tube-shaped hyphae with septa and spherulas. A, Grey matter of the cerebral cortex with infiltration of mucormycosis hyphae and softening of the substrate around the vessel.

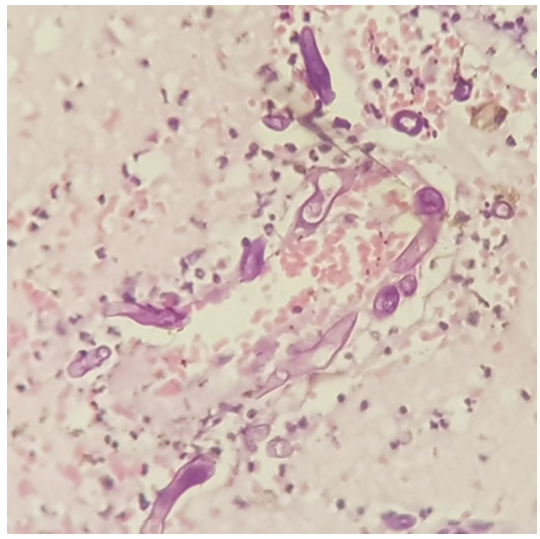


Figure 5b - B, Ring-shaped brain lesion with accumulation of mucormycosis bacilli (hyphae). Hematoxylin and eosin, x 400.

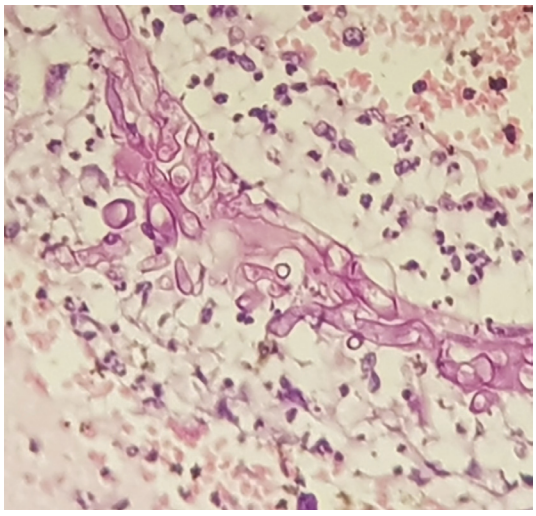


Figure 6a - Photomicrograph of nasal cavity mucosa and eyelid mucosa with mucormycosis lesions. A, Mucormycosis accumulation in the submucosal layer with perivascular localization.

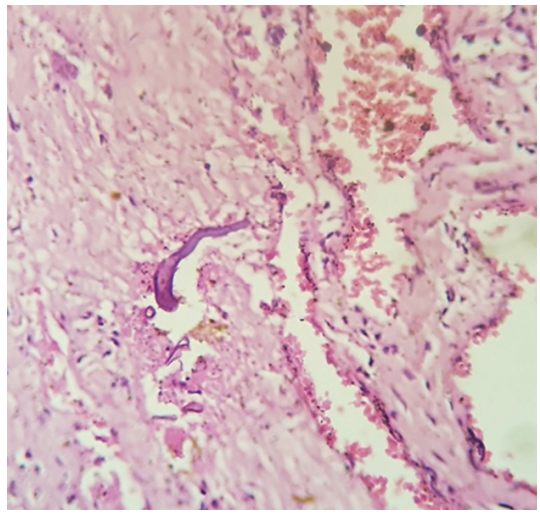


Figure 6b - B, Complete necrosis associated with mucormycosis of the lower eyelid mucosa with accumulation of mucormycosis hyphae.

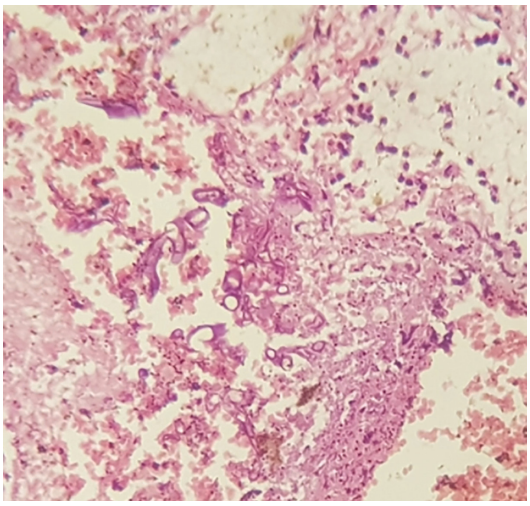


Figure 6c - C, The same region of the lower eyelid in the deeper tissue with accumulation of mucormycosis bacilli.

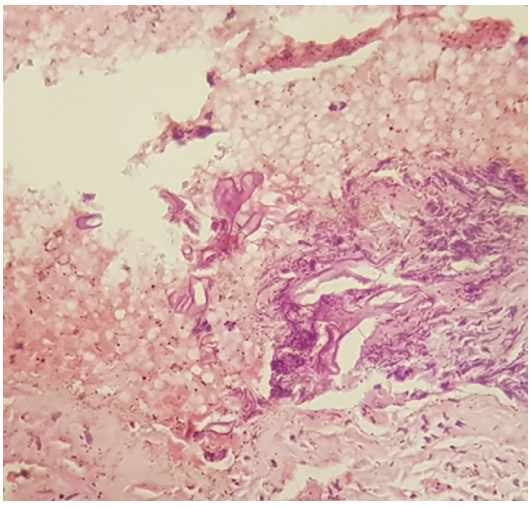


Figure 6d - D, Photomicrograph of a histological examination of necrotic nasal cavity tissue with accumulation of mucormycosis bacilli, mucormycosis fungal hyphae. Hematoxylin and eosin, x200.

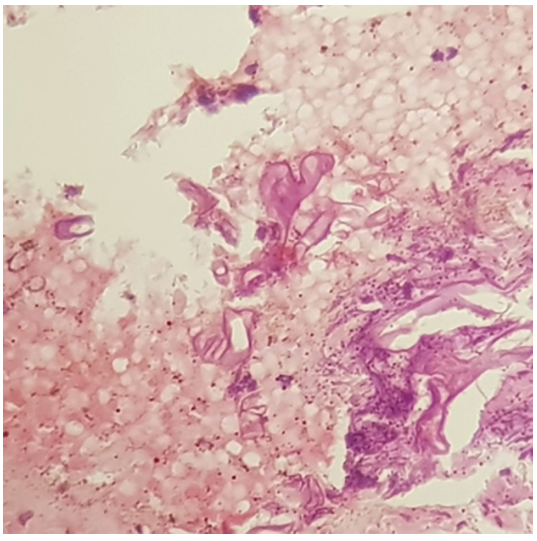


Figure 7a - Lung tissue with ARDS/DAD (diffuse alveolar damage): Masson's bodies and autophages. A, Exudate from mononuclear cells with erythrocytes admixture in lumen of respiratory alveoli with polypoid connective tissue nodule - Masson's bodies with foci of organizing pneumonia. Hematoxylin and eosin, x100.

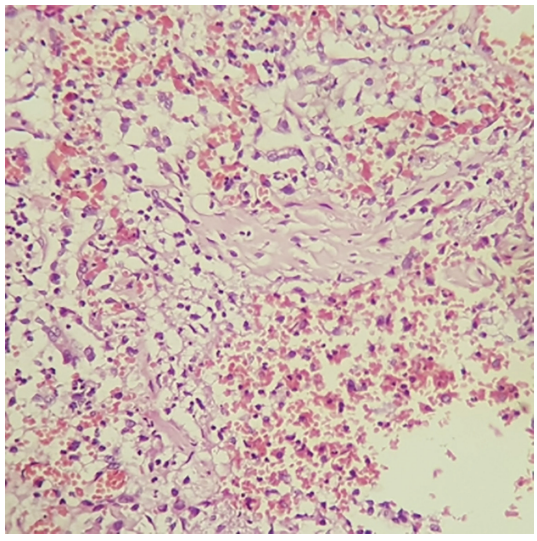
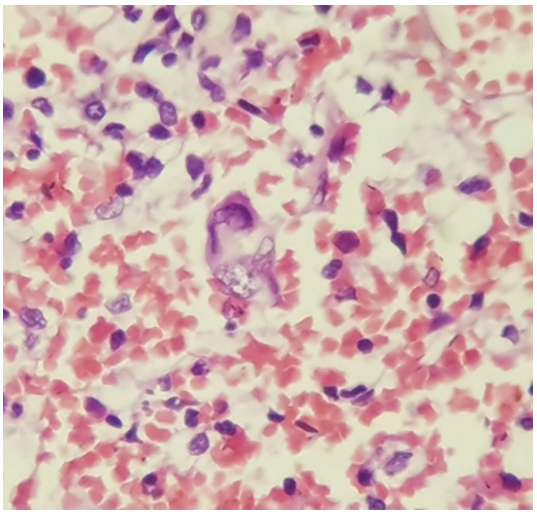
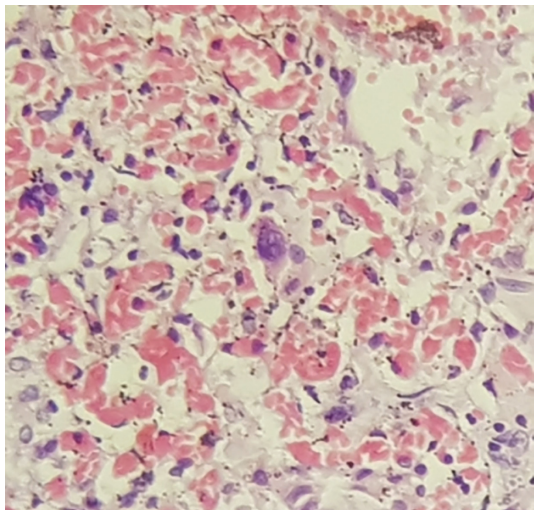


Figure 7b - B, Same region with autophages with viral inclusions in the cytoplasm of alveolar macrophages with hemorrhage. Hematoxylin and eosin, x400.

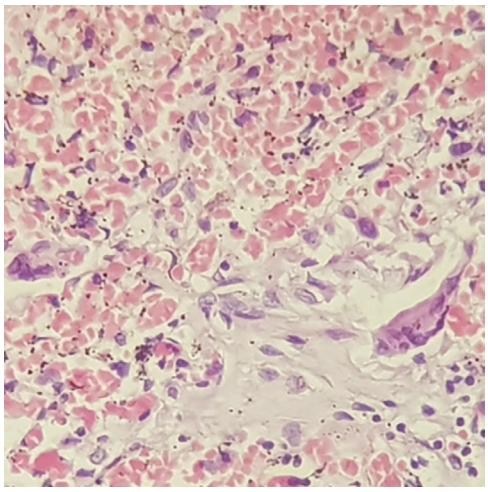
Figure 8a - Monster macrophages are multinucleated like giant cells. A, Multinucleated inclusions in the cytoplasm of autophages are clearly seen as monster macrophages.





Histological examination identified fungal infiltration in the tissues from the nasal septum mucosa, soft tissue of the eye socket, and brain tissue in the form of bamboo sticks, hyphae, spherulas, and septa with inflammatory cellular infiltrates. Photomicrographs of these changes were captured with a Leika DM1000 microscope for scientific and practical purposes (Figures 2-8). Diffuse alveolar damage of lung tissue in the form of Masson's bodies - polypoidal fibrous nodules in the lumen of alveoli was found. Diffuse infiltration of the alveolar lumen by monster macrophages in the form of multinucleated cells due to penetration of viral particles into the cytoplasm of type 2 pneumocytes was observed, which corresponds to acute adult respiratory distress syndrome (ARDS) in the proliferative phase from 10 to 20 days' time from the onset of disease in COVID-19 (Figures 7, 8).

Figure 8b - B, Another region of the lung by giant cell macrophages with mononuclears in the lumen of the respiratory alveoli. Hematoxylin and eosin, x200.



Discussion

In this case, the patient developed coronavirus infection associated with diabetes mellitus. Due to the severe progression, she was prescribed glucocorticosteroids, which exacerbated the severe immunosuppression and led to the development of mucormycosis, even despite her young age. Comparison with data from the literature showed that with timely diagnosis, without manifestation with COVID-19, of this fungal infection in different countries is more effective and timely, as well as treatment, in many cases, patients recovered [7, 9, 10]. While doctors in Kazakhstan had not previously observed mucormycosis associated with COVID-19 in patients with coronavirus infection. Therefore, in described case the doctors, despite the presence of diabetes mellitus, due to the deterioration of the patient's health status, were forced to prescribe glucocorticosteroids for COVID-19 treatment as part of the complex therapy. As other studies also show, the combination of diabetes mellitus and immunosuppression with glucocorticosteroids dramatically increases the risk of mucormycosis [11-13]. Thus, our data have demonstrated that in patients with COVID-19 and comorbid diabetes mellitus, prescription of glucocorticosteroids must be avoided or combined with sensitive antifungal drugs. This opinion also coincides with that of other authors who have observed mostly in India [12]. In general, given the extremely rare confirmed case of mucormycosis in Kazakhstan (1 case), this case is an instructive example for clinicians in the future. Nevertheless, in further cases of severe coronavirus infection associated with comorbidities, every effort must be made to prevent and effectively treat mucormycosis.

An autopsy found specific evidences of macro and microscopic changes specific for mucormycosis, a detailed description of microscopic changes in the internal organs was made, confirmatory photographs were taken, which allowed to exclude oxygen burns of the face in the intensive care unit. Original microscopic photographs of the mucormycosis fungus itself, along with changes in the surrounding tissues and cells, were obtained.

In contrast to other countries, no other cases of this fungal infection, namely mucormycosis with a more favorable progression, were identified in Kazakhstan [14]. This can be explained by the low frequency of mucormycosis associated with COVID-19 coronavirus infection in Kazakhstan in general, relative to other countries, relatively low frequency of cases with a severe disease progression. Also interesting is the fact that this fungal infection developed in a young patient, whereas among older patients there were more fatal outcomes from COVID-19 during the pandemic, but no evidences of mucormycosis were found. It cannot be excluded that some elderly patients with fatal infection could have small foci of mucormycosis without destruction of soft tissue of the face, but not as in our case with facial disfigurement. Therefore, dissemination of the educational information about this extremely dangerous fungal infection, in the case of COVID-19 infection associated with diabetes mellitus, is very important for doctors of different disciplines.

Conclusion

This case allowed a detailed macroscopic, microscopic description and verification of specific evidences of rhino-orbital-cerebral mucormycosis in a young patient, clarification of predisposing factors, and conclusions about the need for timely prevention and treatment of this severe infection.

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