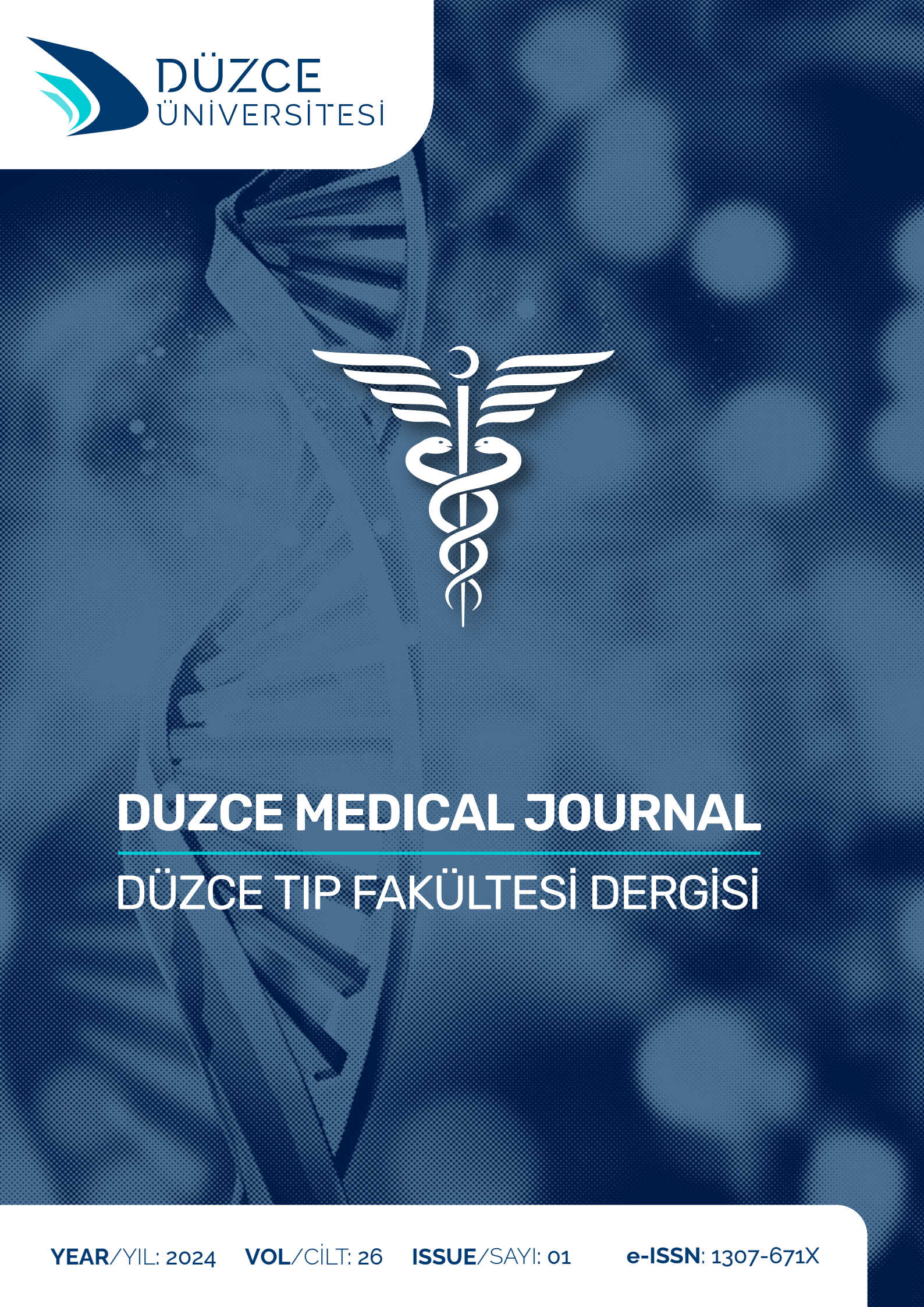

DÜZCE ÜNİVERSİTESİ

DUZCE MEDICAL JOURNAL

DÜZCE TIP FAKÜLTESİ DERGİSİ

YEAR/YIL: 2024 **VOL**/CİLT: 26 **ISSUE**/SAYI: 01 **e-ISSN**: 1307-671X

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CONTENTS / İÇİNDEKİLER

CONTENTS / İÇİNDEKİLER

Editorial / Editöryal
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Duzce Med J, 2024;26(1):1-2
Düzce Tıp Fak Derg, 2024;26(1):1-2

An Important Step on the Road to a High-Impact Journal

Yüksek Etkili Bir Dergiye Giden Yolda Önemli Bir Adım Daha

Mehmet Ali SUNGUR

The recent development of the Duzce Medical Journal, an important step on the road to a high-impact journal, led me to share this news with our readers. This is the first issue of the 26th volume and this editorial was written to share with our readers that the journal has been accepted for inclusion in the Web of Science Core Collection and to briefly summarize the last 5 years of the journal.

An editorial summarizing the first 20 years of Duzce Medical Journal and mentioning its future goals was published in the first issue of the 21st volume. In this editorial, the brief history of the journal was shared and the working plan and future goals of the new editorial board were mentioned. After taking over, the editorial board started making changes by using the submission and manuscript management system and made visual changes such as changing the cover and layout of the articles, as well as important changes such as publishing reviews only by invitation.

In the year 2020, following the publication of this editorial, another dramatic step, changing the publication language of the journal was taken to receive full-texts only in English as of the 22nd volume. Since then, the primary publication language of the journal has been English, the title, abstract, and keywords of the articles are published in both English and Turkish, while the main text is published only in English. In addition, the editorial board, which reviewed and rearranged the author guidelines and writing rules, began to take firmer steps towards high-quality scientific publishing by introducing serious regulations and controls in ethical policy and scientific publishing standards.

Another innovation in this period was the publication of special issues in the journal for the first time, apart from regular issues. Duzce Medical Journal, which was not indifferent to the challenges and developments in the field of medicine and health sciences during the coronavirus disease 2019 (COVID-19) pandemic period, followed all the knowledge and developments closely and introduced to its readers two special issues: "Health Care and Health Management in Pre- and Post-Pandemic Period" in 2020 and "Global Imbalances, Economic and Social Changes, and Health Management in Pre- and Post-Pandemic Period" in 2021. While the first special issue aimed to compile qualified reviews and research articles on medicine and other related areas with an interdisciplinary perspective during and after the COVID-19 pandemic period, the aim of the second special issue was to compile qualified reviews and research articles on global imbalances, economic and social changes, as well as health management and health policy. By 2022, taking into account the increasing number of readers, Duzce Medical Journal, which recognizes its service to science and the scientific community as a debt, has presented a special issue themed "Infertility: Current Concepts" to its readers. The special issue aimed to compile qualified reviews on infertility and related issues, and treatment options in male and female patients with the interdisciplinary perspective of both Urology and Gynecology. Finally, a special issue with the theme of "General Principles and Modeling Techniques in Experimental Animal Studies" has been planned for 2024 in order to emphasize the importance of animal experiments in clinical studies, and the production process of this special issue is in progress.

The number of submissions, which increased about threefold compared to previous periods in the remaining eight-month period after the new editorial board took over in 2018, continued to increase in 2019 and reached twice the number submitted in 2018. In 2020, this number of submissions increased 1.4 times compared to 2019 and continued at this level reached in subsequent years. As of the first quarter of 2024, the number of submissions was the same as last year. Among these submissions, as a result of the preliminary evaluation by the editorial board, and subsequent double-blind peer-review processes, the acceptance rates were 56% in 2018, 37% in 2019, and 36% in 2020 and 2021. As of 2022 and 2023, this rate continues to be 38%.

Another effort of the new editorial board with the aim of leading the authors in improving and revising their manuscripts and publication of the highest quality as much as possible, was to publish informative articles in the field of biostatistics to emphasize the importance of biostatistics in medical research starting from the 3rd issue of the 23rd volume.

Duzce Medical Journal, whose number of international citations has constantly increased as a result of the strict monitoring and hard work of the editorial board along with all the regulations and improvements made in terms of scientific publishing standards, has continuously increased the number of international citations calculated in SCOPUS over the years and reached a total of 123 citations as of 2023 (Figure 1). As a result of the hard work of the editorial board, one of the important goals was achieved and Duzce Medical Journal, which is increasingly recognized internationally, finally received the recognition it deserved at the end of this momentum and was selected to be included in the Web of Science Core Collection. Thus, Duzce Medical Journal, which is indexed in international indexes such as DOAJ, EBSCO, SCOPUS, and also ULAKBİM TR Index as a national index, is now also included in the Emerging Sources Citation Index, adding a new one to the indexes it covered. It has been reported that Duzce Medical Journal will be included in the Emerging Sources Citation Index starting from the 24th volume in 2022 as a result of the evaluation by the Web of Science Editorial Team. With this acceptance, Duzce Medical Journal, which has taken its international influence and recognition one step further, continues its work without slowing down or any interruption. The editorial board continues to work to increase the international recognition of Duzce Medical Journal and to be included in more international indexes.

Finally, with this new development, the journal cover changed to give it a more innovative appearance and in line with the corporate identity culture of the university. Starting from the 1st issue of the 26th volume, the new cover of the journal has begun to use (Figure 2).

As a publication policy, invited reviews, original research articles, and case reports in the field of basic and clinical sciences from the field of general medicine are evaluated for publication in the Duzce Medical Journal. As the editorial board, we approach each study submitted with the awareness that it is the outcome of a process prepared with great effort by the authors, and we strive to finalize the preliminary evaluation as soon as possible, sharing the excitement of the authors. We strive to serve science and scientists with the aim of getting one step better and further than yesterday, without compromising scientific content and quality.

Hereby, I would like to express my sincere thanks to all our reviewers, authors, and readers once again for their interest and contribution to Duzce Medical Journal.

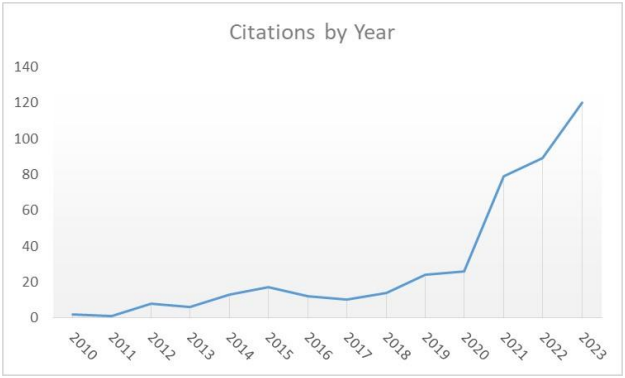

Figure 1. Number of citations by years
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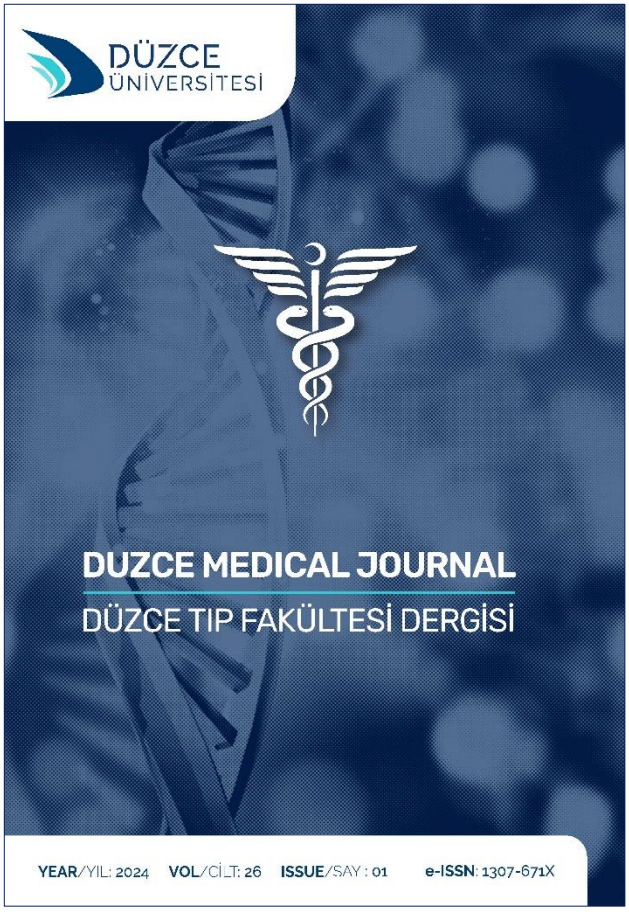

Figure 2. The new cover of the journal since 26th volume

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From the Laboratory to the Clinic: Molecular Treatment of Heart Failure

Laboratuvardan Kliniğe: Kalp Yetmezliğinin Moleküler Tedavisi

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ABSTRACT

Coronary and cardiovascular diseases are the leading cause of death today, with heart failure being among the primary culprits. Heart failure can occur as a result of many diseases, so research in this area is important in terms of clinical outcomes and treatment. Histopathology of heart failure includes cardiac hypertrophy, inflammation, angiogenesis, and apoptosis pathways. The issue of elucidating the pathology of heart failure is still an area of active research. In advanced heart failure, the typical management strategy is medical treatment, mechanical ventricular support devices, and heart transplantation. Heart failure, which occurs with modifiable and non-modifiable risk factors, can be controlled with both non-pharmacological and pharmacological treatment applications. It is especially important to focus on new treatment methods and introduce them to the clinic. Although they are all not yet used in clinics, many studies have yielded promising results with molecular treatment options for heart failure prevention. Studies in animals have shown that heart failure stops proceeding when angiogenesis is induced. Promising results have also been achieved with stem cell therapy, but these may not be implementable for years. It is expected that studies following phases 1 and 2, of the studies which had positive results in the treatment of heart failure, will be conducted and applied in the daily treatment practice.
Keywords: Heart failure; molecular treatment; stem cell therapy.

ÖZ

Koroner ve kalp-damar hastalıkları günümüzün önde gelen ölüm nedenidir; kalp yetmezliği ise primer etiyoloji arasında yer almaktadır. Kalp yetmezliği birçok hastalığın getirisi olarak ortaya çıkabildiğinden bu alanda yapılacak araştırmalar klinik sonuçlar ve tedavi açısından önemlidir. Kalp yetmezliğinin histopatolojisi, kalp hipertrofisi, inflamasyon, anjiyogenez ve apoptoz yolaklarını içerir. Kalp yetmezliği patolojisinin aydınlatılması konusu halen aktif bir araştırma alanıdır. İlerlemiş kalp yetmezliğinde tipik tedavi stratejisi tıbbi tedavi, mekanik ventriküler destek cihazları ve kalp naklidir. Değiştirilebilen ve değiştirilemeyen risk faktörleriyle ortaya çıkan kalp yetmezliği hem farmakolojik hem de non-farmakolojik tedavi uygulamalarıyla kontrol altına alınabilmektedir. Özellikle yeni tedavi yöntemlerine odaklanılması ve bunların kliniğe tanıtılması önemlidir. Henüz klinikte kullanılmamasına rağmen, birçok çalışma kalp yetmezliğinin önlenmesine yönelik moleküler tedavi seçenekleriyle umut verici sonuçlar vermiştir. In vivo yapılan çalışmalar, anjiyogenez uyarıldığında kalp yetmezliğinin ilerlemesinin durduğunu göstermiştir. Kök hücre tedavisinde de umut verici sonuçlar alınmakta iken aktif uygulama yapılamamaktadır. Kalp yetmezliği tedavisinde olumlu sonuç alınan çalışmalardan faz 1 ve 2. aşamayı takip eden çalışmaların yapılması ve günlük tedavi pratiğinde uygulanması beklenmektedir.
Anahtar kelimeler: Kalp yetmezliği; moleküler tedavi; kök hücre terapi.

INTRODUCTION

The most common cause of death in the United States (US) is heart disease. The life expectancy for females was 5.0 years higher than for males, and the life expectancy at birth was 78.6 years (1). Heart failure (HF) is a significant cause of morbidity and mortality. In 2016, there were 6.2 million HF patients in the US, and by 2030, this rate is expected to increase by 46% (2).

According to European Society of Cardiology (ESC) guidelines for acute and chronic HF, HF is defined as follows (3): "A clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling, and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles, and peripheral edema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress." The etiology of HF is varied and includes myocardial, pericardial, endocardial, heart valve disease, vascular disease, and metabolic disease (4).

Advanced HF, cardiogenic shock, and related deaths often occur despite optimal medical therapy (β-blockers, angiotensin-converting enzyme inhibitors, mineralocorticoid receptor antagonists, and implantable cardioverter defibrillator (ICD) treatments). Symptoms should be noted, and congestion status should be assessed at each examination. Symptoms associated with fluid retention usually regress when treated with diuretics. Findings such as increased jugular venous pressure and displacement of the apex beat are more specific, but they may be difficult to detect, and their applicability is low (5,6). The most common symptoms of HF are dyspnea, orthopnea, paroxysmal nocturnal dyspnea, decreased exercise intolerance, fatigue, prolonged recovery time after exercise, and ankle edema. Typical findings of this are increased jugular venous pressure, hepatojugular reflux, 3rd heart sound (gallop rhythm), and lateral displacement of the apex beat.

It may be more difficult to identify symptoms and signs of HF in obese patients, elderly patients, and patients with chronic lung disease, so the symptom status should be monitored after treatment. HF is life-threatening, decreases the quality of life, is high in comorbidity, brings a significant economic burden to the health system, and is a progressive disease that often leads to long hospital stays, expensive mechanical devices, heart transplantation, and/or death. (7,8). Coronary artery disease is among the most common risk factors of HF, so controlling HF necessitates early diagnosis, control, and follow-up for coronary artery disease (9-11).

The aim of this review was to describe molecular treatment methods that depict myocyte loss in cases of HF in cardiovascular risk groups apart from routine treatment methods.

TREATMENT METHODS

Research areas are directed to both non-pharmacological and pharmacological management of HF, at the same time, close monitoring of the patient at home with various devices is also considered (12,13).

The most common cardiovascular disease is coronary artery disease. Many risk factors cause coronary artery disease, these are modifiable and non-modifiable factors. Correction of modifiable risk factors and lifestyle change are very important. Blood pressure control, lipid therapy, physical activity, diet, and stopping smoking are among modifiable reasons. (14-17).

Since both genetic and lifestyle features predispose to coronary artery disease, genomic sequencing, and analytical technologies provide patients the opportunity to lipid control strategy individually. Although it is not widely used in the clinic, it is an active research area to determine the genetic risk score by identifying the DNA variants causing cardiovascular disease. The individual risk can be identified, and susceptibility to disease can be understood and individualized strategies can be applied before the development of the disease to avoid or delay the disease (18-20).

β-blockers, angiotensin-converting enzyme inhibitor drugs, mineralocorticoid receptor antagonists, diuretics, and angiotensin receptor neprilysin inhibitors are recommended with strong indication to reduce the risk of hospitalization and death due to HF in symptomatic patients (21).

In patients with advanced HF, device therapy methods reduce the risk of sudden death of the patient, prolong life, and the use of ICD, cardiac resynchronization therapy-defibrillator (CRT-D) is recommended with definite indication despite the cause of HF being ischemic or non-ischemic the power which the heart required to spend is reduced, organ perfusion increases, and more oxygen is delivered to the tissues (22).

The gold standard treatment for advanced HF is heart transplantation. According to International Society for Heart and Lung Transplantation (ISLTH) records, from 1982 to 2012, more than 100,000 heart transplant operations were performed worldwide. The 1-year survival rate is 81%, the 5-year survival rate is 69%, and the median survival rate is 11 years (23).

Heart transplantation performed in end-stage HF provides long-term successful survival. Unfortunately, not all patients meet the criteria for heart transplantation and the problem of donor insufficiency continues. Besides, supportive therapy and rehabilitation are required. Cardiac rehabilitation is an evidence-based practice and patient education, and behavior modification are important for the secondary prevention of cardiovascular diseases (24).

MOLECULAR MECHANISMS IN HEART FAILURE

New insights into the pathophysiology and molecular mechanisms of HF are required to develop new therapeutic approaches. To overcome HF, some advances in understanding the molecular pathways associated with cardiac hypertrophy, inflammatory signaling (e.g. TNF-α, IL-6), and oxidant stress that may play an important role in modifying transcriptional regulatory networks which regulate adaptation or non-compliance should be identified. In addition to paracrine mechanisms (VEGF, CCN1) and intracellular signaling (IL-6-glycoprotein 130), the effects of current treatment options on these molecular pathways and the potential effects on cardiac failure progression should be clarified (25,26).

Molecular events occurring in pathological hypertrophy are characterized by activation of gene expression patterns. It is a fetal stage that generally includes the up-regulation of fetal isoforms of genes that regulate cardiac contractions

and calcium uptake which is mostly parallel with the down-regulation of adult isoforms (e.g. up-regulation b-MHC; down-regulation α-MHC). More recently, evidence has been presented that shows that pathophysiological stresses also affect normal cell transformation in the heart, leading to a negative rate of cardiac apoptosis and regeneration from circulating cardiac progenitor cells (27). In the last decade, great progress has been seen in understanding the molecular mechanisms of adaptive and maladaptive hypertrophy and HF in response to stress signals, and the involvement of several extracellular factors and signaling pathways (28).

Firstly, understanding the important role of neurohormonal activation in the pathophysiology of HF has led to the improvement of morbidity and mortality in the current medical treatment of patients with HF. Accordingly, recent experimental data support the concept that aldosterone blockade provides beneficial effects in addition to effective renin-angiotensin system blockade. Aldosterone is one of the stimulants for the production of cardiomyocyte reactive oxygen species (ROS) that play a role in the development of cardiac hypertrophy and dysfunction in response to both biomechanical and neurohormonal stimuli. Numerous studies have reported that ROS (superoxide, hydrogen peroxide, hydroxyl radical) increases myocardial production in experimental and clinical HF. After two weeks of treatment, angiotensin II-induced cardiac hypertrophy was related to NAD(P)H oxidase activation. Recent in vitro and in vivo studies have provided evidence that the antioxidant effects of statins have an important role in cardiac hypertrophy and vascular dysfunction in patients with HF (29,30).

It is well known that increased TNF-α levels occur in the circulation of patients with HF. In a study, a mouse with a transgene overexpressing TNF developed cardiac hypertrophy, and dilated cardiomyopathy indicated that this cytokine plays a deleterious role in the heart. Also, TNF-α exerts a strong direct effect on cardiomyocytes since it causes apoptosis in cardiomyocytes, depression of contractility, and in vitro downregulation of sarcomeric proteins (31).

Recently, IL-6-gp130-Janus kinase (JAK)-STAT signal cascade was investigated in patients with end-stage HF, and it was demonstrated that the pathway of this mechanism changes at all levels in people with HF (32). Although these studies did not identify the exact role of individual factors in the IL-6-gp130-JAK-STAT signaling system, it was identified with increasing experimental models that IL-6-associated cytokine signaling contributes to compensatory hypertrophy, ensures heart protection, and promotes neovascularization in the stressed heart (33).

In HF, the conversion of mechanical stress to biomechanical signals is thought to be largely mediated by a group of surface receptors called integrins. Furthermore, intracellular signaling pathways regulated by the melusin with integrin sensor have been shown to contain ERK1/2, PI3-K/Akt, and glycogen synthase kinase 3-beta (GSK-3). Gene mutations that cancel one of these pathways result in early HF in response to inadequate hypertrophic response and mechanical stress. Activation of these two signaling pathways is thought to be important for promoting adaptive hypertrophy and preventing failure during early dilatation and hemodynamic overload (34,35).

Failure to induce adequate neovascularization results in inadequate oxygen supply followed by loss and degeneration of cardiomyocytes, atrophy, and interstitial fibrosis, and may represent the main cause of myocardial dysfunction and HF. STAT3 plays a very important role among the signal molecules involved in the expression of proangiogenic factors in cardiomyocytes. Both STAT3 and JunD regulate the expression of proangiogenic secreted factor VEGF, pointing to the key role of this protein in postnatal myocardial angiogenesis, in which cardiomyocytes themselves play an important role as a source for VEGF. A second proangiogenic factor, CCN1 is induced in the heart in vivo and in cardiomyocytes in vitro by various extracellular stress-related stimuli such as neurohumoral activation, cytokines, mechanical stress, and ischemia (36).

GATA4, which is a cardiac-enriched zinc transcription factor, plays an important role in both cardiac hypertrophy and myocardial angiogenesis. GATA4 is abundantly expressed in cardiomyocytes at early embryonic stages, regulates cardio-specific gene expression, and is downregulated in adult hearts. HIf-1, on the other hand, is a transcription factor that is stabilized under hypoxic conditions, and transactivates angiogenesis-related proteins such as VEGF and erythropoietin in the hypoxic medium.

As is known, cardiac neovascularization is not dependent on a single gene or factor but is based on the regulation of multiple factors by various signaling pathways. In patients with HF, new treatment strategies that promote the endogenous secretion of angiogenic factors have been introduced by directly applying proangiogenic factors to increase neovascularization (37,38).

Apoptosis plays a role in cardiomyocyte cell loss during the development of HF. Apoptosis rates between 0.08% and 0.25% in patients with end-stage dilated cardiomyopathy are between 0.001 and 0.002% in control hearts. Activation of caspase-8 is a central step in apoptosis initiated by activation of cell surface death receptors (e.g. Fas/FasL). In the study conducted by Wencker et al. (39) in 2003, procaspase-8 treated with a broad-spectrum caspase inhibitor prevented before dilatation and impaired cardiac dysfunction that began before cardiac decompensation in transgenic mice. Caspase inhibition and inhibition of cardiomyocyte death were found to be significant; apoptosis was reduced, and treatment success was increased. Also, although drug therapies targeting signaling agents that induce apoptosis such as B-adrenergic receptor blockers and angiotensin II inhibitors are standard in HF therapy, apoptotic pathways seem to have direct effects on cardiomyocyte contractility and remodeling (40).

Recent research has shown that cardiac hepatocyte growth factor/insulin-like growth factor-1 signaling plays a crucial role in cardiac regeneration for the migration of cardiac stem cells into the heart and their proliferation and differentiation (41).

MOLECULAR TREATMENT OPTIONS

Further studies are needed to determine the optimal combination of angiogenic growth factors and improve the technology of myocardial administration methods to increase the efficacy and safety of therapeutic

interventions for myocardial angiogenesis. In a rat model of myocardial infarction, the combination of fibroblast growth factor-2 and hepatocyte growth factor prevented the progression of HF by synergistically inducing angiogenesis. Cardiomyocytes themselves produce angiogenic factors to maintain capillary density, oxygen supply, and function. Besides, in endothelial cells, short activation of Akt alleviates the damage caused by ischemia, while long-term activation of Akt leads to unorganized blood vessel formation similar to tumor vasculature (42).

In another study, copper supplementation reversed contraction dysfunction and prevented the transition to HF in pressure-overloaded mice, partly through the promotion of myocardial angiogenesis (43). In addition to cobalt and copper function, several approved drugs have been reported to affect myocardial angiogenesis. For example, pitavastatin has been reported to induce myocardial angiogenesis and prevent the progression of HF. Although the promotion of myocardial angiogenesis needs much more work before becoming a prescribed drug for HF patients, a certain amount of preclinical evidence has accumulated and the applying this concept in clinical practice will likely continue to progress steadily in the coming years (44).

In a study in which cardiomyocyte efficacy was tested, it was shown that transplanted fetal cardiomyocytes can survive in the scar tissue of the heart, limiting scar expansion and preventing HF (45).

Given these previous studies, what are the functional roles of p53 accumulated in HF? The two main roles of P53 are cell cycle arrest and prevention of blood vessel formation. In cases of severe cellular damage, p53 arrests proliferative cells in the G1 stage of the cell cycle to induce apoptosis or aging. Although cardiac myocytes do not proliferate after birth, the accumulation of p53 causes apoptotic cell death in cardiac myocytes. In vivo, chemical inhibition of p53 accumulation or transcriptional activity mitigated adriamycin-induced cardiomyopathy in heart and cardiac failure after myocardial infarction. Although it is certain that neurohumoral factors, mechanical and oxidative stresses, metabolic changes, and cardiac dysfunction accompanied by DNA damage have accompaniment, definite triggers and mechanisms for the disruption of coordinated angiogenesis remain unclear (46).

DISCUSSION

Increased ROS production is involved in key issues related to the development and progression of HF, such as cardiomyocyte hypertrophy, ventricular dysfunction, and endothelial dysfunction. Statin therapy is a treatment that can have beneficial effects on HF by reducing oxidative stress and increasing endothelial nitric oxide availability.

An in-depth understanding of the molecular mechanisms of HF will provide valuable insights for the design of new treatment strategies that support protective signaling pathways and prevent incompatible responses such as advanced hypertrophy, inadequate vascularization, and apoptosis. Some of these findings may have important effects on the development of new treatment strategies in HF (47).

Mesenchymal stem cells are non-hematopoietic cells that have the potential to differentiate into various cell types. They were initially diagnosed in the bone marrow, but are also found in umbilical cord blood, adipose tissue, and heart. The use of these cells in mouse myocardial infarction models has led to the development of remodeling and the reduction of infarct size after their differentiation into cardiomyocyte and endothelial phenotypes (48).

The application strategies of stem cells are transvascular approaches and direct injection into the LV wall. Intracoronary conduction, intravenous infusion, and mobilization of stem cells are among these strategies. It has been reported that stem cells initiate myocardial repair and improve heart function with direct and indirect mechanisms including differentiation into the heart and vascular cells, paracrine effects, and cell fusion. The paracrine effect is a concept used for the therapeutic effects of transplanted stem cells on injured tissues. Transplanted stem cells release cytokines, chemokines, growth factors, exosomes, or microparticles, repair damaged myocardium, and stimulate changes that initiate restoration processes in the extracellular matrix (49,50).

The most obvious question to be answered by preclinical studies is which type of stem cell or progenitor cell is the most suitable candidate for treatment. The potential for regeneration of bone marrow-derived progenitor cell therapy under prescribed conditions (acute myocardial infarction) has been controversial but proved to be safe and beneficial. Cardiac stem cells have the potential to be patient-specific, but isolation and culture procedures are at an early stage of development. Embryonic stem cells have the potential to differentiate but face ethical barriers and also have the greatest risk of teratoma formation. The survival and integration of transplanted cells can also be improved by placing them in matrices such as collagen or matrigel, placing cells in monolayered layers, or simultaneously transmitting growth factors (51).

Today, new gene transfer therapy protocols for cancer and some genetic diseases are also applied to strengthen angiogenesis and perform recovery faster, especially in ischemic heart diseases. Especially in recent years, SERC2A gene transfer made with adenoviruses, a commonly used viral vector, has helped to achieve important results by regulating calcium metabolism in HF patients (52).

Until now the number of drug groups, whose positive effects on both quality of life and prognosis in the treatment of HF are demonstrated, is still very small. Cardiac transplantation, dynamic cardiomyoplasty, Batista and Dor operations, left ventricular assist devices, total artificial heart, biventricular "pacing" and ultrafiltration are the other treatment methods used in the clinic (53).

CONCLUSION

In the treatment of HF, positive preliminary results were obtained and phase 1 and phase 2 studies began to be conducted in humans; methods such as cellular cardiomyoplasty (increasing endogenous and exogenous myocyte cells), gene therapy, regulation of cellular calcium metabolism, prevention of apoptosis, angiogenesis induction are expected to be applied in the daily treatment practice.

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Proportion of Colonic Diverticulosis and Its Associated Factors among Patients Underwent Colonoscopy

Kolonoskopi Yapılan Hastalarda Kolonik Divertikülozis Oranı ve İlişkili Faktörler

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ABSTRACT

Aim: Diverticular disease is one of the most common gastrointestinal disorders to date, with a notable rising trend in developing countries. However, the proportion of colonic diverticulosis and its associated factors among patients who have undergone colonoscopy remains controversial. This study aimed to determine the local data on the proportion of diverticular disease in the community, its complications, the association of diverticulosis with diabetes mellitus and hypertension, as well as demographic characteristics.
Material and Methods: A retrospective review of medical records was performed among patients who had undergone colonoscopy between January and December 2019. Demographic and clinical characteristics, the presence of diabetes mellitus, hypertension, and diverticular disease and its complications were examined, and the association of diverticular disease and its complications with demographic and clinical characteristics were analyzed.
Results: Out of 221 patients, 12.7% (n=28) of them had diverticular diseases with a slightly predominant right-sided occurrence (42.9%, n=12). There were significant associations with age (p=0.002), ethnicity (p=0.011), and hypertension (p=0.036), but not with gender and diabetes mellitus (p=0.261, and p=0.334, respectively). There was no significant association between hypertension and recurrence of complicated diverticulitis (p=0.741), septic complications (p=0.678), and diverticular bleeding (p=0.243). Diabetes mellitus was significantly associated with diverticular bleeding complications (p=0.001) but not with septic complications (p=0.418) and recurrence of complicated diverticulitis (p=0.629).
Conclusion: This study showed almost a similar percentage of diverticulosis compared to previous local studies. Age, ethnicity, and hypertension were associated with the presence of diverticulosis, and diabetes mellitus was associated with diverticular bleeding.
Keywords: Diverticular disease; age; ethnicity; hypertension; diabetes mellitus.

ÖZ

Amaç: Divertiküler hastalık bugüne kadar en sık görülen gastrointestinal hastalıklardan biridir ve gelişmekte olan ülkelerde belirgin bir artış eğilimi göstermektedir. Ancak kolonoskopi yapılan hastalarda kolonik divertikülozis oranı ve bununla ilişkili faktörler tartışmalıdır. Bu çalışmada, toplumdaki divertiküler hastalık oranına ilişkin yerel verilerin, komplikasyonları, divertikülozisin demografik özelliklerin yanı sıra diyabet ve hipertansiyon ile ilişkisinin de incelenmesi amaçlandı.
Gereç ve Yöntemler: Ocak ve Aralık 2019 tarihleri arasında kolonoskopi yapılan hastaların tıbbi kayıtları geriye dönük olarak incelendi. Demografik ve klinik özellikler, diyabet, hipertansiyon ve divertiküler hastalık varlığı ve komplikasyonları incelendi ve divertiküler hastalık ve komplikasyonlarının demografik ve klinik özellikler ile ilişkisi analiz edildi.
Bulgular: 221 hastanın %12,7'sinde (n=28) divertiküler hastalıklar mevcuttu ve hafif baskın olarak sağ tarafta (%42,9, n=12) görülüyordu. Yaş (p=0,002), etnik köken (p=0,011) ve hipertansiyon (p=0,036) ile anlamlı ilişki vardı, ancak cinsiyet ve diyabet ile anlamlı ilişki yoktu (sırasıyla p=0,261 ve p=0,334). Hipertansiyon ile komplike divertikülit nüksü (p=0,741), septik komplikasyonlar (p=0,678) ve divertiküler kanama (p=0,243) arasında anlamlı bir ilişki saptanmadı. Diabetes Mellitus'un divertiküler kanama komplikasyonları (p=0,001) ile anlamlı düzeyde ilişkili olduğu ancak septik komplikasyonlar (p=0,418) ve komplike divertikülit nüksü (p=0,629) ile ilişkili olmadığı görüldü.
Sonuç: Bu çalışma önceki yerel çalışmalarla karşılaştırıldığında neredeyse benzer bir divertikülozis yüzdesi gösterdi. Yaş, etnik köken ve hipertansiyon divertikülozis varlığıyla, diyabet ise divertiküler kanamayla ilişkiliydi.
Anahtar kelimeler: Divertiküler hastalık; yaş; etnik köken; hipertansiyon; diyabet.

INTRODUCTION

Diverticular disease is one of the most common gastrointestinal disorders to date, where it is most prevalent in Western countries due to dietary habits, with a notable rising trend in developing countries. Despite being known to have less prevalence of colonic diverticular disease, the adoption of the Western diet into the Asian population may have led to an increment in the prevalence of cases (1). Diverticular disease is thought to be a disease of the elderly with a proportionate increment with age, where more than 60% of affected individuals are older than 80 years old, and only 10% of them are younger than 40 years old. However, the incidence of diverticular disease among younger populations appears to be arising. This poses a diagnostic challenge as 70% of these young patients require surgical intervention, whereas most of the elderly patients remain asymptomatic throughout their lives.

Many aspects of diverticular disease remained uncertain, making primary and secondary prevention seem impossible. Previous theories on the pathophysiology of diverticular disease support that of bacterial overgrowth causing infection, but recent reports indicate an inflammatory process initiated by the release of pro-inflammatory cytokines, as evidenced by the abundance of mast cells seen at all layers of histopathological sections of the diseased colonic wall. Other causes include alterations in colonic wall resistance, disordered colonic motility, and dietary deficiencies, especially fiber (2). This pro-inflammatory state is present in diabetes mellitus patients, whereas hypertension is associated with a worse prognosis for patients to develop complicated diverticular disease due to the increased pressure in blood vessels causing vascular endothelial injury and subsequently atheroma formation, leading to arteriosclerosis. This, in turn, will cause the affected blood vessels in the diverticula to be more fragile; elevating the risk of them to be ruptured leading to bleeding complications (3).

Recent studies have shown the association between metabolic disorders and colonic diverticular disease. The surge in the prevalence of hypertension and diabetes mellitus in the Malaysian population may contribute to an increase in the prevalence of colonic diverticulosis. Hypertension and diabetes mellitus patients are common in the Malaysian population, where the number of Malaysians suffering from both diseases increases proportionately with age. A similar trend is observed with the prevalence of diverticulosis. Several years have passed but the percentage of hypertensive patients in the Malaysian population has been in a plateau (around 30%) since 2011. Diabetes mellitus, on the other hand currently affects 1 in every 5 adults in Malaysia, with its prevalence on the rise as reported by NHMS 2019; 11.2% in 2011, 13% in 2015 to 18.3% in 2019 (4). A 2012 Israeli study reported that diabetes mellitus serves as a protective factor against the development of diverticulosis (5), contradicting a Japan-based study which found that more concomitant diabetes mellitus is discovered among patients with diverticulum than those without diverticulum (6), supported by a 2011 Malaysian-based study which reported that diabetes mellitus is associated with recurrent complicated diverticular disease (7). The same Israeli study also concluded that arterial hypertension is not related to diverticulosis whereas the same Japanese study found that more diverticulum patients have hypertension compared to those with no diverticulum.

Only 10-25% of patients have symptoms, thus the diagnosis of diverticular diseases is often incidentally detected by investigations of the lower gastrointestinal tract (8). Symptoms range from non-specific abdominal pain and feeling bloated to complications such as acute diverticulitis, bleeding, and perforations, which are also signs and symptoms for numerous other gastrointestinal disorders (2). Although most patients with complicated diverticular disease are self-remitting and have a generally good prognosis, they are at risk of disease recurrence and eventually need surgical intervention.

Diagnostic tools used to determine the presence of diverticulosis include CT colonography and/or CT abdomen, colonoscopy, barium enema, and ultrasonography; where all are with different sensitivity and specificity. CT colonography and/or abdomen ranked first, especially in diagnosing acute diverticulitis.

In this study, we aimed to obtain local data on the proportion of diverticulosis in the community, its complications, the association of diverticular disease to diabetes mellitus and hypertension, as well as demographic characteristics; and subsequently compare it with other regional statistics.

MATERIAL AND METHODS

This study is a retrospective review of the medical records in the Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan, and Hospital Raja Perempuan Zainab II (HRPZ II), Kota Bharu Kelantan from January to December 2019 involving all patients who had underwent colonoscopy that fulfilled the inclusion and exclusion criteria.

The list of patients was obtained from the colonoscopy room via admission records. During the whole span of 2019, a total of 862 colonoscopies were conducted in the HRPZ II while 770 colonoscopies were done in HUSM. Systematic random sampling where every 7^{th} record was chosen to be selected as subject (9). Details were then filled up into data collection forms (pro forma). The subjects were each assigned with unique subject ID and pro forma filled were kept in files only accessible by the investigator.

A total of 221 patients' records that fulfilled the criteria were enrolled in the study. Subjects are labeled with study code to maintain privacy and confidentiality. The inclusion criteria for this study included patients aged above 18 years old who underwent colonoscopy from January to December 2019. Cases with incomplete data of colonoscopy findings from records, incomplete colonoscopy, and patients who had undergone prior bowel resection were excluded.

The sample size was calculated using a single proportion formula for the first objective and two independent proportion formulas for the second objective, using PS: Power and Sample Size Calculation Version 3.0.4.3. To determine the proportion of colonic diverticular disease in Kelantan from January until December 2019, the sample size was determined based on parameter estimates obtained from Wong et al. (10).

For the second objective, which is to determine the factors associated with colonic diverticulosis in Kelantan from January until December 2019, the sample size was calculated using the parameter estimates obtained from Rajendra et al. (11) with a significance level of 0.05, and the power of the study of 80%. The final targeted sample size was determined by considering a 20% drop-out rate. The estimated sample size for this study was 221.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the Human Research Ethics Committee, Universiti Sains Malaysia in Kelantan, Malaysia (18.11.2020, USM/JEPeM/20080442).

Statistical Analysis

The data were descriptively analyzed in mean and standard deviation (SD) or median and interquartile range (IQR) for continuous data. For categorical data, frequency and percentage were used. The chi-square test and Fisher's exact test were used to determine the association between colonic diverticulosis and its complications with hypertension and diabetes mellitus, as well as patients' demographics, where appropriate. Data analyses were performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). The level of significance was set at 0.05.

RESULTS

A total of 221 samples were randomly selected by systematic random sampling method which were enrolled from the colonoscopy records of both tertiary hospitals. The mean age of patients was 57.1±16.9 (range, 20-89) years, where the majority of patients (n=116, 52.5%) were >60 years old. Of the patients, 132 (59.7%) were male. The majority of the patients were Malay (n=186, 84.2%). This is consistent with demographic data of Kelantan, Malaysia population where more than 90% of the population is Malay. Hypertension and diabetes mellitus were present in 49.3% (n=109) and 28.1% (n=62) of the patients, respectively. Colonic diverticulosis was detected in 12.7% (n=28) of the study group. There was a slight predominance of right-sided diverticulosis (n=12, 42.9%) as compared to left-sided occurrence 35.7% (n=10), whereas 21.4% (n=6) have bilateral diverticulosis. (Table 1).

In those older than 60 years of age, 19.8% (n=23) were diagnosed with diverticulosis during colonoscopy. None of the patients aged younger than 40 years old have diverticulosis on colonoscopy, while only 7.8% (n=5) of the 41-59 age group have diverticular disease. There was a significant association between age and presence of diverticulosis (p=0.002).

Among the 89 female patients, 15.7% (n=14) were diagnosed with diverticulosis while only 10.6% (n=14) suffered from diverticulosis among males (Table 2). However, there was no statistically significant association between gender and the presence of diverticulosis (p=0.261).

While 18 (9.7%) of the Malay patients had diverticulosis, 10 (30.3%) Chinese had diverticulosis, whereas none of the Indians and other ethnicities had the disease. Statistical analysis indicated that race was significantly associated with the presence of diverticulosis (p=0.011).

Of the 109 patients with hypertension, only 19 (17.4%) had diverticulosis, while those without diverticulosis comprised 90 (82.6%) patients. In 112 non-hypertensive patients, only 9 (8%) were diagnosed with diverticulosis, whereas 103 (92%) patients had no diverticulosis. There was a significant association between hypertension and the presence of diverticulosis (p=0.036).

While 10 (16.1%) of the patients with diabetes mellitus had diverticulosis, 52 (83.9%) of these patients did not. Among 159 non-diabetic patients, only 18 (11.3%) of them suffered from diverticular disease, whereas 141 (88.7%) patients were free of it. However, there was no statistically significant association between diabetes mellitus and the presence of diverticular disease (p=0.334).

Among the hypertensive patients, 21.1% (n=4) had a history of acute diverticulitis prior to colonoscopy, while 15.8% (n=3) had recurrent complicated diverticulitis after colonoscopy (Table 3). Similar findings were also noted in the non-hypertensive group. There was no statistically significant association between hypertension and recurrence of complicated diverticular disease (p=0.741).

A similar finding was also noted among the diabetic and non-diabetic group of patients. Among the 20% (n=2) of diabetic patients with a previous history of acute diverticulitis, 10% (n=1) was found to have recurrent complicated diverticulitis after colonoscopy, while among the 22.2% (n=4) of non-diabetic with previous acute diverticulitis, 16.7% (n=3) developed complicated diverticulitis after colonoscopy (Table 3). However, there was no statistically significant association between diabetes mellitus and recurrence of complicated diverticular disease (p=0.629).

Hypertension was also not associated with septic complications (p=0.678), bleeding complications (p=0.243), or any complications of diverticular disease (p=0.483). Bleeding complications were present in 31.6% (n=6) of the hypertensive patients and 15.8% (n=3) had septic complications.

While 7 (70%) diabetic patients suffered from complicated diverticular disease, and 27.8% (n=5) of non-diabetics had the same disease (Table 3). Statistical analysis indicated that diabetes mellitus was significantly associated with

Table 1. Patient demographics and clinical characteristics

	All Patients (n=221)
Age (years), mean±SD	57.1±16.9
Age, n (%)	
<40 years	41 (18.6)
41-59 years	64 (29.0)
>60 years	116 (52.5)
Gender, n (%)	
Male	132 (59.7)
Female	89 (40.3)
Ethnicity, n (%)	
Malay	186 (84.2)
Chinese	33 (14.9)
Indian	1 (0.5)
Others	1 (0.5)
Hypertension, n (%)	109 (49.3)
Diabetes mellitus, n (%)	62 (28.1)
Diverticular disease, n (%)	28 (12.7)
Anatomical distribution of diverticular disease, n (%)	*(n=28)*
Left	10 (35.7)
Right	12 (42.9)
Bilateral	6 (21.4)

SD: standard deviation

Table 2. Presence of diverticular disease according to the patient demographics and clinical characteristics

	Age			p
	<40 years (n=41)	**41-59 years (n=64)**	**>60 years (n=116)**	
Presence of diverticular disease, n (%)	0 (0.0)	5 (7.8)	23 (19.8)	**0.002**

	Gender		p
	Male (n=132)	**Female (n=89)**	
Presence of diverticular disease, n (%)	14 (10.6)	14 (15.7)	0.261

	Ethnicity				p
	Malay (n=186)	**Chinese (n=33)**	**Indian (n=1)**	**Others (n=1)**	
Presence of diverticular disease, n (%)	18 (9.7)	10 (30.3)	0 (0.0)	0 (0.0)	**0.011**

	Hypertension		p
	Yes (n=109)	**No (n=112)**	
Presence of diverticular disease, n (%)	19 (17.4)	9 (8.0)	**0.036**

	Diabetes mellitus		p
	Yes (n=62)	**No (n=159)**	
Presence of diverticular disease, n (%)	10 (16.1)	18 (11.3)	0.334

Table 3. History of acute diverticulitis before colonoscopy, recurrent complicated diverticulitis after colonoscopy, septic and bleeding complications, and complicated diverticular disease according to hypertension and diabetes mellitus status

	Hypertension		p	Diabetes Mellitus		p
	Yes (n=19)	**No (n=9)**		**Yes (n=10)**	**No (n=18)**	
History of acute diverticulitis, n (%)	4 (21.1)	2 (22.2)	0.944	2 (20.0)	4 (22.2)	0.891
Recurrent complicated diverticulitis, n (%)	3 (15.8)	1 (11.1)	0.741	1 (10.0)	3 (16.7)	0.629
Septic complication, n (%)	3 (15.8)	2 (22.2)	0.678	1 (10.0)	4 (22.2)	0.418
Bleeding complication, n (%)	6 (31.6)	1 (11.1)	0.243	6 (60.0)	1 (5.6)	**0.001**
Complicated diverticular disease, n (%)	9 (47.4)	3 (33.3)	0.483	7 (70.0)	5 (27.8)	**0.030**

diverticular complications (p=0.030). Septic complications refer to acute diverticulitis and complicated diverticulitis such as diverticular abscess, perforation, and fistulas. When separated into septic and bleeding complications, only the bleeding complication is significantly associated with diabetes mellitus (p=0.418, and p=0.001, respectively). 10% (n=1) of diabetic patients have septic complications and 60% (n=6) have bleeding complications, whereas 22.2% (n=4) of non-diabetics suffered from septic complications, and only 5.6% (n=1) have bleeding complications.

DISCUSSION

The result of this study showed that 12.7% of patients were diagnosed with diverticular disease in 221 colonoscopy records examined. This is a slight increment compared to an earlier Malaysian-based study conducted in 2005 at a private institution which had a 10% prevalence of diverticular disease diagnosed using colonoscopy (11). Otherwise, this study showed a lower prevalence when compared to two Japanese studies that were conducted over 20 years (from 1990 to 2010) with a reported 18.8% prevalence (12), and 20.3% prevalence for a study conducted from 2003 to 2011 (13). On the other hand, another study in Japan conducted from 1965 to 1980 had a lower percentage (7.8%) of prevalence in the initial year that increased up to 12.3% during the last 3 years of the study (14). A Singaporean study showed a 45% prevalence of diverticular disease diagnosed with barium enema from the year 2001 to 2002 (15), a rising number as compared to an earlier study in a similar setting which reported a prevalence of 28% in the year 1988 till 1989 (16). An Indian-based study reported a 9.9% prevalence of diverticular disease. Western part of the world showed a prevalence of 5 to 10% in young age groups and this can increase up to 70% in the elderly (17).

Anatomic distribution of the colonic diverticular disease can be divided into the right (cecum, ascending, and transverse colon), left (entire colon distal to splenic flexure), or bilateral involvement (11). Of the 28 patients with colonic diverticulosis in our study, 10 (35.7%) were left-sided, 12 (42.9%) were right-sided and 6 (21.4%) were bilateral. There is a slight predilection to the right side of about 42.9% compared to the left side of 35.7%, although left-sided diverticular disease is still considered common. Various Asian-based studies have a right-sided diverticulum preponderance as compared to our Western counterparts (7). This finding is comparable to a study made in Brunei where the reported cases of right-sided diverticulum were 37.1%, left-sided diverticulum of 32.7%, and bilateral diverticulum of 26.1% (10). This is further supported by a study in Thailand using an analysis of barium enema involving a sample of 2877 subjects where a significant right-sided predominance was reported, with double the amount compared to left-sided diverticulum (18).

A Malaysian study conducted in 2010 reported a recorded number of 24 out of 121 patients (19.8%) having recurrent complicated diverticular diseases with a significant association with diabetes mellitus. The present study has shown that 10% of diabetic patients developed recurrent complicated disease after colonoscopy. A possible reason

for this is due to diabetes mellitus being known to cause pro-inflammatory conditions, delay wound healing, affect microvasculature circulations in the intestines, and various other systemic effects; where all these factors may have contributed to this finding (7). However, we did not include the data on diabetes mellitus control in this study group, which would have had effects on developing more complications related to diverticular disease.

Most of our study group were elderly patients, where this group of subjects are likely associated with more comorbidities and poorer general health, and therefore, may have a less favorable outcome following an acute attack of diverticulitis.

This study found that ethnicity was related to the presence of colonic diverticulosis. However, Indians and other ethnicity (Siamese, in this study) only constituted 1% of the study group and none of them were diagnosed with diverticular disease. Therefore, it is difficult to conclude the actual frequency among different racial groups.

Colonoscopy performed in the elderly can have its unique challenges which include poor bowel preparations, multiple comorbidities resulting in higher hypotensive episodes with the use of sedatives secondary to prolonged procedures, and a lesser number of completed colonoscopy procedures (19). As most of our study group was in the elderly group, this carries a risk of missing the presence of diverticular disease in the study group as an incomplete procedure has to be excluded from this study.

Complicated colonic diverticular disease can be seen as bleeding, acute diverticulitis, or segmental colitis (20,21). These complications can cause morbidity and mortality. One aspect we looked into was the association between the recurrence of complicated colonic diverticular disease with the presence of metabolic conditions; hypertension and diabetes mellitus. Our finding reported no association both hypertension and diabetes mellitus with the recurrence of complicated colonic diverticular disease. This result was supported by a previous study that reported a significant association between diabetes mellitus and the recurrence of complicated diverticular disease, and no significant association with hypertension (7). This study could probably be improved further by studying the population admitted to the ward for complicated colonic diverticular disease with a bigger sample size.

The present study reported a non-significant association between hypertension with septic or bleeding complications of colonic diverticular disease. However, a prospective case-control study on colonic diverticular bleeding conducted in Japan concluded that hypertension has an odds ratio (OR) of 2.2 to cause diverticular bleeding, thus considered an independent risk factor (3). The basis of risk factors for bleeding diverticular are conditions that are known to alter blood flow and angioarchitecture (22,23).

There is a significant association between diabetes mellitus and complicated diverticular disease (24), mainly towards bleeding diverticular (25). These results are not in accordance compared to a study that noted diabetes mellitus has no significant association with diverticular bleeding (26). Diabetes mellitus is associated with an immunocompromised state as it impairs blood circulation and adversely affects normal immunological response. However, in this study diabetes mellitus has no significant association with septic complications of diverticular disease. It is interesting to note that a study reported that obesity (a risk factor for diabetes mellitus) is associated with an increased incidence and severity of complicated diverticular disease, which is diverticulitis (27).

Our study is limited also by the absence of a sub-analysis of the severity of the comorbidity; hypertension and diabetes mellitus. We understood that some of the patients with multiple comorbidities would be on polypharmacy. This limitation hinders us from concluding the association of risk of bleeding and sepsis especially those with uncontrolled diabetes mellitus and those on anticoagulant or antiplatelet therapy. Perhaps in future studies, we could collect more detailed variables regarding this to perform a sub-analysis on the outcomes related to the severity of the comorbidities and medications towards the proportion of diverticulosis and its complications.

CONCLUSION

We conclude that this study reported almost similar percentage of diverticular disease compared to other previous local studies and that there were significant associations between age, ethnicity, and hypertension with the presence of diverticular disease but hypertension is not significantly associated with either septic or bleeding complications of diverticular disease. Diabetes mellitus is associated with diverticular bleeding. However, the presence of diabetes mellitus, as well as gender, are not significantly associated with diverticular disease. Some studies showed similar results but there are also contradicting outcomes, suggesting that further, more comprehensive study is needed to be done in the future. Understanding the connection between these comorbidities and diverticulosis allows healthcare professionals to more accurately assess the risks and benefits before initiating multidrug therapy. For example, since diabetes mellitus is associated with bleeding complications, it is crucial to advise patients that the initiation of anticoagulants or antiplatelets puts them at a higher risk of bleeding. This informed approach enables healthcare providers to make more precise decisions tailored to the individual's health profile.

Ethics Committee Approval: The study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (18.11.2020, USM/JEPeM/20080442).

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Detection of Carbapenem Resistance Using the Genotypic and Phenotypic Methods in *Klebsiella pneumoniae*

Klebsiella pneumoniae Suşlarında Karbapenem Direncinin Genotipik ve Fenotipik Yöntemler ile Saptanması

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ABSTRACT

Aim: This study aimed to detect the carbapenem resistance of the *Klebsiella pneumoniae* strains, isolated from clinical specimens with genotypic and phenotypic methods.
Material and Methods: A total of 87 *Klebsiella pneumoniae* strains whose carbapenem resistance was determined by disc diffusion method were included in the study. Carbapenemase was investigated using the combined disk method and polymerase chain reaction (PCR).
Results: The evaluation of the PCR results demonstrated that OXA was detected in 60 (68.9%) samples, NDM was detected in 20 (22.9%), OXA + NDM in 5 (5.7%), and KPC was detected in 1 (1.1%) out of 87 clinical samples. Carbapenemase was not detected in one specimen with the PCR method. The results were found compatible with the combined disc test results for all isolates which were detected as only OXA, NDM, and KPC type carbapenemase positive. In 5 (5.7%) strains in which the co-existence of NDM and OXA type carbapenemases was detected by PCR, the combined disc method detected only OXA type carbapenemase.
Conclusion: The combined disk method is inadequate in the presence of strains that have multiple carbapenemases, and also have OXA which is the most frequently detected carbapenemase in our hospital. EUCAST recommends verification by other methods in the presence of OXA-48. Genotypic methods can be used for confirmation testing. The detections of strains with NDM, multiple carbapenemases, and the first detection of KPC were striking in the study. Monitoring the spread of these strains in the hospital will be necessary for infection control.
Keywords: *Klebsiella pneumoniae*; carbapenem; carbapenemase.

ÖZ

Amaç: Bu çalışmada, klinik örneklerinden izole edilen *Klebsiella pneumoniae* suşlarında karbapenem direncinin genotipik ve fenotipik yöntemler ile saptanması amaçlanmıştır.
Gereç ve Yöntemler: Disk difüzyon yöntemi ile karbapeneme dirençli bulunan toplam 87 *Klebsiella pneumoniae* suşu çalışmaya dahil edilmiştir. Karbapenemaz varlığı kombine disk yöntemi ve polimeraz zincir reaksiyonu (polymerase chain reaction, PCR) ile araştırılmıştır.
Bulgular: PCR sonuçları değerlendirildiğinde, 87 klinik örnekten 60 (%68,9) örnekte OXA tipi, 20 (%22,9) örnekte NDM tipi, 5 (%5,7) örnekte OXA + NDM tipi ve 1 (%1,1) örnekte ise KPC tipi karbapenemaz saptandığı görülmektedir. Bir örnekte ise PCR yöntemi ile karbapenemaz bulunamamıştır. Tek başına OXA, NDM ve KPC tipi karbapenemaz pozitifliği saptanan izolatların tamamı için sonuçların kombine disk testi ile uyumlu olduğu bulunmuştur. PCR yöntemi ile NDM ve OXA tipi karbapenemazın birlikte olduğu 5 (%5,7) örnekte ise kombine disk yönteminde sonuç OXA tipi karbapenemaz olarak bulunmuştur.
Sonuç: Kombine disk yöntemi, aynı anda birden fazla karbapenemaz bulunduran suşların bulunması ve hastanemizde en sık saptanan karbapenemaz tipinin OXA olması nedeni ile yetersiz kalmaktadır. EUCAST, OXA-48 varlığında diğer yöntemlerle doğrulanmasını önermektedir. Doğrulama testi olarak genotipik yöntemler kullanılabilir. NDM tipi karbapenemazın artmakta olduğu, birden fazla karbapenemazı taşıyan suşların görülmeye başlaması ve ilk defa KPC tipi karbapenemazın bulunması dikkat çekicidir. Bu suşların hastanede yayılımının takip edilmesi enfeksiyon kontrolü açısından önemli olacaktır.
Anahtar kelimeler: *Klebsiella pneumoniae*; karbapenem; karbapenemaz.

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INTRODUCTION

Antibiotic resistance has recently become a serious public health problem worldwide, and gram-negative bacteria have an important role in this with their various antibiotic resistance mechanisms. Antibiotic resistance has become widespread, mainly owing to the extensive and inappropriate use of antibiotics (1,2).

Carbapenems are a group of beta-lactam antibiotics that have rapid bactericidal activity and the broadest spectrum. In recent years carbapenem-resistant *Klebsiella pneumoniae* (CRKP) strains have frequently been isolated in different regions of the world. Carbapenem-resistant *Klebsiella pneumoniae*-associated infections lead to the prolongation of hospital stay, together with higher mortality, and morbidity (3,4).

The minimum inhibitory concentration (MIC) values of carbapenems may vary depending on the type, and level of the carbapenemase enzyme and on the bacteria type. Therefore, the rapid and accurate detection of carbapenemase-producing bacteria has significant importance for the selection of the appropriate antimicrobial treatment and the application of infection control procedures (5).

Beta lactamases are divided into four molecular classes from A to D according to their amino acid structure (6). NMC- IMI, SME, GES and *Klebsiella pneumoniae* carbapenemase (KPC) are the four families of the Class A carbapenemases. KPC has emerged as a critical carbapenemase from gram-negative bacteria mostly from *K. pneumoniae* in the world (7). The differentiation of ESBL and AmpC from KPC is difficult when they are together with porin loss/change (8,9).

Besides, automated systems give inconsistent results in the detection of KPC-producing bacteria (3). Class B carbapenemases, also known as metallo-β-lactamases, include VIM, NDM, IMP, and NDM-1 (New Delhi metallo-β-lactamase) (10). NDM-1 was first identified in Sweden in 2008 from the *K. pneumoniae* strain isolated from a patient with a history of hospitalization in India then was reported in the USA, the United Kingdom, and many other countries especially related to traveling to India, and Pakistan (11,12). Class D consists of OXA-type carbapenemases. The OXA-48 was first reported from Turkey in 2003 (13). The enzyme is endemic in Turkey, Morocco, Libya, Egypt, and Tunisia (14).

Phenotypic and genotypic methods can be used in the detection of carbapenemases. Chromogenic media, modified Hodge test, inhibitor-based methods (double disk synergy, combined disc tests), biochemical methods, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), or immunochromatographic methods can be used as the phenotypic method. The polymerase chain reaction (PCR), sequencing, and oligonucleotide hybridization can be used as genotypic methods. Molecular methods are recommended for fast and accurate detection of carbapenemases. Phenotypic methods can be used in laboratories where molecular tests cannot be performed (5,15).

We aimed to investigate and perform the typing of the carbapenem resistance in *Klebsiella pneumoniae* strains isolated from the clinical specimens of the inpatients or the patients who applied to the outpatient clinic after being discharged using the phenotypic and genotypic methods in the present study. The obtained results will provide valuable information for appropriate antimicrobial therapy and infection control.

MATERIAL AND METHODS

Throat swab, rectal swab, tracheal aspiration, bronchoalveolar lavage, hemoculture, urine, sputum, drainage fluid, pleural fluid, and abscess samples of inpatients who were admitted to the outpatient clinic of Istanbul University Istanbul Medical Faculty Hospital between 2015 and 2017 were evaluated. A total of 87 isolated *Klebsiella pneumoniae* strains that had been found resistant to carbapenem by the disk diffusion method were included in the study. No clinical discrimination was performed, and examples from all services were included in the study. A single clinical sample of each patient was included in the study.

Isolates were identified using conventional methods. After morphologic examination, Gram staining was performed on suspicious colonies, and the identification of the Gram-negative rods was performed using the catalase, oxidase, motion examination, VP reaction, citrate, indole, urea, H2S formation, lysine, and ornithine decarboxylase tests. Antibiotic susceptibility was investigated using the disk diffusion method (Oxoid-United Kingdom) according to the recommendations of the Clinical and Laboratory Standards Institute (16).

The combined disc method (D70C, Mast Group, United Kingdom) was used as the phenotypic method in the detection of carbapenemase. Meropenem (10 μg), meropenem (10 μg) + MBL inhibitor, meropenem (10 μg) + KPC inhibitor, meropenem (10 μg) + AmpC inhibitor, and temocillin (30 μg) discs (TEM30C, Mast Group, United Kingdom) were used in the test.

Two different real-time multiplex PCR kits were used as the genotypic method: MDR KPC / OXA Real-TM (Sacace, Italy) kit KPC and OXA-48/162 (with no distinction), MDR MBL (VIM, IMP, NDM Real-TM (Sacace, Italy) kit identify the VIM, IMP, and NDM.

All procedures were approved by the ethical standards of the Ethics Committee of the Istanbul University Istanbul Faculty of Medicine (25.11.2016, 1355).

Statistical Analysis

The IBM SPSS v.21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.) program was used for statistical analysis of the data. In descriptive statistics, number and percentage values were calculated. The chi-square test was used for comparison between groups. The results were evaluated at the 95% confidence interval. A p value of <0.05 was considered as statistical significance.

RESULTS

Thirty-seven carbapenem-resistant strains from 2015, 26 from 2016, and 24 from 2017 were evaluated. The samples were taken during the successive period. No significant difference was detected between the years in the evaluation of the enzyme results ($p=0.153$).

The evaluation of the PCR results demonstrated that OXA-48/162 was detected in 60 (68.9%) samples, NDM was detected in 20 (22.9%) samples, OXA-48/162 + NDM in 5 (5.7%) samples, and KPC was detected in 1 (1.1%)

sample out of 87 clinical samples. No enzyme was detected in one specimen in the PCR and combined disc method.

This strain was found resistant only to ertapenem. All isolates that were detected as single OXA, NDM, and KPC type carbapenemase with PCR were found compatible with the combined disc test results. In five (5.7%) samples where the coexistence of NDM and OXA type carbapenemase were determined with PCR, the result was found as OXA type carbapenemase in the combined disk method.

The investigation of the distribution of the OXA and NDM positive 80 samples showed that OXA positivity was found in 3 (42.9%) strains in the neonatal intensive care unit, 7 (100%) strains in anesthesiology and reanimation, and 7 (100%) strains in cardiovascular surgery clinics. The statistical investigation showed that the lower detection of OXA positivity in the neonatal intensive care unit, and higher in the anesthesiology and reanimation, and cardiovascular surgery units were significant (p=0.031). The distribution of carbapenemases by years is shown in Figure 1, and the distribution of the clinics is shown in Figure 2.

The antibiotic susceptibility test results of our study showed that 3 (5%) strains were susceptible to cefotaxime, 6 (10.7%) were susceptible to ceftazidime, 7 (10.6%) strains were susceptible to cefepime, and 1 (1.5%) strain was intermediately susceptible to cefepime. No carbapenemase was detected in the tests performed on the strain which was intermediately susceptible to cefepime. OXA-type carbapenemase was detected in all strains that were found susceptible to one or more of cefepime, cefotaxime, and ceftazidime.

Tobramycin resistance was detected as 100% in the strains with NDM and OXA+NDM and 74% in the strains with OXA. Gentamicin resistance was detected as 87.8% in the strains with NDM and OXA+NDM, and 66% in the strains with OXA. Amikacin resistance was detected as 75% in the strains with NDM and OXA+NDM, and 26.6% in the strains with OXA. Three (6.1%) strains were intermediately susceptible to amikacin.

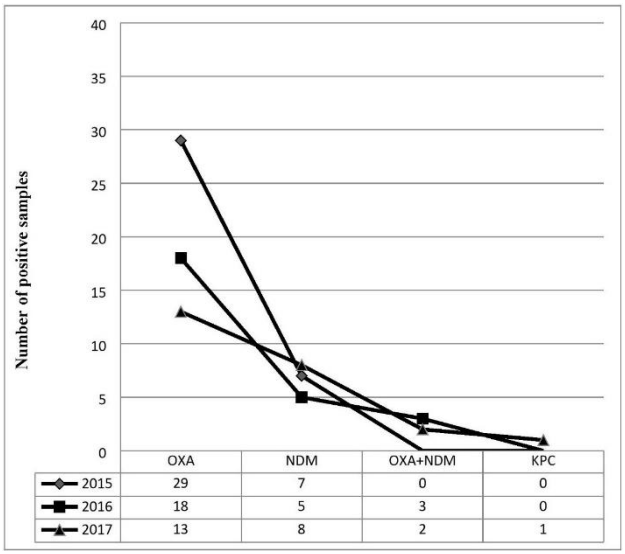

Figure 1. The distribution of carbapenemases by years

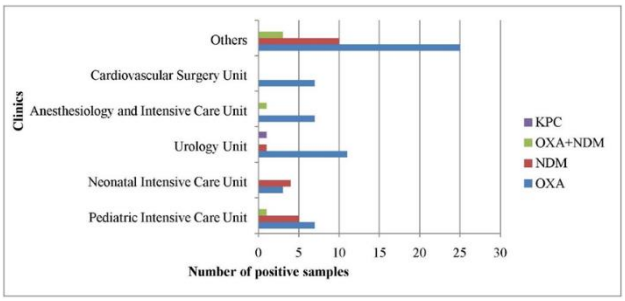

Figure 2. Distribution of carbapenemases by clinic

DISCUSSION

There has been a rapid spread of antibiotic resistance worldwide in recent years. Immediate and accurate detection of carbapenemase-producing bacteria is particularly crucial in the treatment of severe, life-threatening infections and infection control.

Molecular methods are recommended for rapid, and accurate detection of carbapenemases. Phenotypic methods can be used in laboratories where molecular tests cannot be performed (5,15). The sensitivity and specificity of the combined disc method were determined as 78-100%, and 93-100% depending on the carbapenemase type of the isolates (15,17).

PCR and combined disc tests were compatible in detecting the carbapenemase in isolates that had OXA, NDM, and KPC only. No enzyme was found in one sample through the use of PCR and combined disc method. This strain was found resistant to ertapenem only. The sensitivity of ertapenem was very high in carbapenemase screening; however, its specificity was lower.

In five (5.7%) samples where the coexistence of NDM and OXA-type carbapenemase was determined with PCR, the result was found as OXA-type carbapenemase in the combined disk method. The detection of the co-existence of carbapenemase enzyme types has been detected at increasing rates (18-20).

OXA-48 was found in the *K. pneumoniae* 11978 strain, isolated in the Hospital of Istanbul Faculty of Medicine in 2001. OXA-48 was suggested to originate and spread from Turkey to the world (13). OXA-48 is the most frequently detected carbapenemase enzyme in Turkey and is also endemic in Morocco, Libya, Egypt, and Tunisia.

We found 60 (68.9%) out of 87 carbapenem-resistant *K. pneumonia* strains as OXA-48 or OXA-162 positive in our study. The distribution of OXA within the years was 80% in 2015, 69% in 2016, and 54% in 2017. The results might be in OXA-48 predominance because OXA-48 was first detected in our hospital. The ratio of OXA-type carbapenemase is consistent with similar studies conducted in Turkey (18,21-23). The decrease in the last two years was found insignificant. However, in two different studies conducted in our hospital in the following years, OXA-type carbapenemase rates were found to be 16% and 25.8% (24,25). It is seen that OXA-type carbapenemase rates continued to decrease in the following years.

NDM-1 was first identified in the *K. pneumoniae* strain in Sweden in 2008 in a patient who was previously hospitalized in India (12). The first NDM-1-positive *K. pneumoniae* strain in Turkey was reported in 2011 (26). In two studies conducted in Istanbul in 2014-2016 and 2015-2016 years,

NDM-type carbapenemase was found to be 22.6% and 27%, respectively (27,28). In a study conducted on *Escherichia coli* and *Klebsiella pneumoniae* strains in Turkey in 2019, the rate of NDM-1 alone was found to be 15% (21).

In our study, 20 (22.9%) out of 87 carbapenem-resistant *K. pneumoniae* strains were found NDM positive. The NDM rate was 19.4% in 2015, 19.2% in 2016, and 33.3% in 2017. Changes in NDM rates by years were not found statistically significant. In two different studies conducted in our hospital in the following years, NDM rates were found to be 36% and 27.7% (24,25). Our NDM-type carbapenemase ratio in these two studies seems to be consistent with the data from 2017.

The co-existence of OXA-48 and NDM-1 was first reported in 2013 in Istanbul (29). In the same year, this association rate was found to be 1% in Kayseri (19). It was found to be 2.1% in a study conducted in 2014 with *E.coli* and *K. pneumoniae* strains, and 6.7% in another study conducted in 2016-2017 (18,23). In a prospective, multicentre observational cohort study conducted in Turkey in 2018-2019, the association of OXA-48-like and NDM was found to be 16% (30). In two studies conducted in our hospital in 2021 and 2021-2022, this rate was found to be 36% and 27.7% (24,25). The co-existence of OXA and NDM was found in five (5.7%) strains in our study. The evaluation of the distribution within years showed that no OXA + NDM co-existence was detected in 2015. The coexistence of OXA + NDM was detected in three (11.5%) out of 26 samples in 2016 and in two (8.3%) out of 26 samples in 2017. This result was consistent with the Turkish data. When evaluated together with the studies conducted in the following years, it is seen that the association of OXA + NDM has increased over the years.

The most common type of class A carbapenemases is KPC which was first detected in 1996 in a *Klebsiella pneumoniae* strain isolated in the United States (31). KPC is endemic in the United States, Greece, and Italy (32,33) KPC could not be identified in most studies conducted in Turkey (18,23,34-37). The first KPC enzyme was reported by Labarca et al. (26) in a KPC-2 expressing *Klebsiella pneumoniae* strain in Turkey in 2014.

In a study conducted in 2015-2016, KPC was found to be 1.1%, and in another study in 2019, it was found to be 16% (21,28). We detected KPC in 2017 in an isolate obtained from a clinical sample of a 62-year-old woman who underwent renal transplantation ten years ago. KPC was shown to become sensitive after gene losses following the repeat culture passages of the carbapenemase-carrying strains. Negative results may be detected if these strains are overlooked (38).

Since KPC has not been previously reported, its spread should be carefully monitored in our hospital. The most common responsible mechanism for the carbapenem resistance of Enterobacteriaceae species is the production of carbapenemase enzymes. The decrease in carbapenem susceptibility may also be detected in the association of GSBL or AmpC enzyme production and porin loss (39). We found no such isolation in our study. In two studies in 2018 and 2019, KPC and NDM associations were detected, but in our study, we did not detect KPC and NDM associations (21,40).

Class D carbapenems hydrolyze penicillins and carbapenems, however, their ability to hydrolyze the extended-spectrum cephalosporins such as cefotaxime, ceftazidime, ceftriaxone, and cefepime may be limited (13). Besides, these strains are rarely susceptible to broad-spectrum cephalosporins owing to the mostly co-existence of OXA-48, and ESBL (41,42).

The evaluation of the antibiotic susceptibility test results showed that 3 (5%) strains were susceptible to cefotaxime, 6 (10.7%) strains were susceptible to ceftazidime, 7 (10.6%) strains were susceptible to cefepime, and 1 (1.5%) strain was intermediately susceptible to cefepime. The strain with intermediate cefepime susceptibility was found susceptible to imipenem and meropenem, and resistant to ertapenem, and no carbapenemase was detected in the conducted tests. OXA-type carbapenemase was detected in all strains that were found susceptible to one or more of cefepime, cefotaxime, and ceftazidime.

The clinical efficacy of the aminoglycosides in carbapenemase-producing strains was reported insufficient, even if they found susceptible in vitro (33). Most strains producing NDM-type carbapenemase are resistant to aminoglycosides because they also include the aminoglycosides inactivating 16S rRNA methylases (43). In our study, the aminoglycoside resistance was found higher in the strains with NDM compared to the strains with only OXA.

CONCLUSION

Although the combined disc method is practical and cost-effective, it is insufficient in strains containing more than one carbapenemase and in the presence of OXA-48 because temocillin resistance is not specific to OXA-48 type carbapenemases. In this case, EUCAST recommends verification by other methods (44). Genotypic methods can be used for confirmation testing. The detection of strains with NDM, multiple carbapenemases, and the first detection of KPC were striking. Monitoring the spread of these strains in the hospital will be necessary for infection control.

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Comparison of Rapid Antibiotic Susceptibility Test Method Directly from Blood Culture Bottle with Standard Disc Diffusion Method

Kan Kültürü Şişesinden Doğrudan Yapılan Hızlı Antibiyotik Duyarlılık Testi Yönteminin Standart Disk Difüzyon Yöntemi ile Karşılaştırılması

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ABSTRACT

Aim: Early determination of antimicrobial susceptibility of sepsis pathogens is important. In this study, we aimed to compare the standard disc diffusion method with the rapid antimicrobial susceptibility testing (RAST) method performed directly from blood culture bottles.

Material and Methods: Bacteria isolated from samples that gave a positive signal on the blood culture device between April 2019 and September 2019 were included in the study, and antimicrobial susceptibilities were determined by the standard disc diffusion method and the RAST method. Categorical agreement, small error, large error, very large error, and area of technical uncertainty ratios were recorded.

Results: A total of 103 bacteria including 19 *S. aureus*, 10 *Enterococcus spp*. and 24 *E. coli*, 24 *K. pneumoniae*, 13 *P. aeruginosa*, and 13 *A. baumannii* were included in the study. When the RAST method was compared with the standard disc diffusion method, 100% agreement was found between the methods against imipenem, meropenem, gentamicin, and trimethoprim-sulfamethoxazole in *E. coli* isolates at all hours evaluated, and against meropenem in *K. pneumoniae* isolates at the 6th and 8th hour. For *S. aureus* and *P. aeruginosa* isolates, very major errors were found in the RAST results. For *A. baumannii* isolates, 100% agreement between methods was observed for many antibiotics.

Conclusion: It was concluded that the RAST method is a simple and inexpensive test for life-threatening infections such as sepsis. It was also felt that similar studies should be carried out with a large number of isolates, as compliance rates vary depending on the bacteria tested.

Keywords: Bacteremia; disc diffusion antimicrobial tests; blood culture.

ÖZ

Amaç: Sepsis etkenlerinin antimikrobiyal duyarlılıklarının erken belirlenmesi çok önemlidir. Bu çalışmada, standart disk difüzyon yöntemi ile kan kültür şişelerinden doğrudan yapılan hızlı antibiyotik duyarlılık testi (HADT) yönteminin karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Çalışmaya Nisan 2019 ile Eylül 2019 tarihleri arasında kan kültürü cihazında pozitif sinyal veren örneklerden izole edilen bakteriler dahil edilmiş ve antimikrobiyal duyarlıkları standart disk difüzyon yöntemi ve HADT yöntemi ile belirlenmiştir. Kategorik uyum, küçük hata, büyük hata, çok büyük hata ve teknik belirsizlik alanı oranları kaydedilmiştir.

Bulgular: Çalışmaya 19'u *S. aureus*, 10'u *Enterococcus spp*. ile 24'ü *E. coli*, 24'ü *K. pneumoniae*, 13'ü *P. Aeruginosa* ve 13'ü *A. baumannii* olmak üzere toplam 103 adet bakteri dahil edilmiştir. HADT yöntemi ile standart disk difüzyon yöntemi karşılaştırıldığında, *E. coli* izolatlarında imipenem, meropenem, gentamisin ve trimetoprim-sülfametoksazole karşı değerlendirilen tüm saatler için, *K. pneumoniae* izolatlarında ise meropeneme karşı 6. ve 8. saatler için yöntemler arasında %100 uyum bulunmuştur. *S. aureus* ve *P. aeruginosa* izolatlarında ise HADT sonuçlarında çok büyük hata saptanmıştır. *A. baumannii* izolatlarında birçok antibiyotik için yöntemler arasında %100 uyum olduğu görülmüştür.

Sonuç: HADT yönteminin sepsis gibi hayatı tehdit eden enfeksiyonlar için kullanımı kolay ve ucuz bir test olduğu sonucuna varılmıştır. Test edilen bakteriye göre değişen uyum oranları nedeniyle benzer çalışmaların çok sayıda izolatla yapılması gerektiği de düşünülmüştür.

Anahtar kelimeler: Bakteriyemi; disk diffüzyon antimikrobiyal testleri; kan kültürü.

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INTRODUCTION

Accurate detection and rapid reporting of bloodstream infections are the two most important functions of the clinical microbiology laboratory (1). Bacteremia can lead to serious complications, including sepsis (2). Sepsis increases morbidity and mortality rates, particularly in patients who spend long periods in intensive care. To prevent this, urgent initiation of broad-spectrum antimicrobial treatment is mandatory (3,4). Identifying bacteria from positive bottles and performing antibiotic susceptibility testing takes 24-48 hours using standard methods. This leads to delays in treatment (5).

The most commonly used antimicrobial susceptibility testing method in clinical microbiology laboratories is disc diffusion, described by Bauer et al. (6) in 1966. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommended direct and rapid antimicrobial susceptibility testing (RAST), which requires a short incubation period from positive blood culture bottles for the major antimicrobials used in the treatment of sepsis. The method is based on the standard EUCAST disc diffusion method but with modified inoculum and incubation time. Undiluted blood culture water from the positive blood culture bottle was used as inoculum and the incubation time was shortened to 4, 6, and 8 hours. The antimicrobials tested were selected to cover the most important agents for the treatment of sepsis (5).

The aim of this study was to perform RAST according to EUCAST recommendations on blood culture bottles with the preliminary diagnosis of bacteremia and giving positive signals and to compare the results with the standard disk diffusion method.

MATERIAL AND METHODS

Blood culture samples sent to the Düzce University Faculty of Medicine Hospital Medical Microbiology Laboratory from different hospitals and outpatient clinics between April and September 2019 were included in the study. Microorganisms isolated from the samples that gave a positive signal on the BACTEC automated blood culture device (Becton Dickinson, USA) were identified by conventional methods and/or the VITEC 2 Compact® system (Biomerieux, France), antimicrobial susceptibilities were tested by the standard disc diffusion method and the results were recorded (7). Blood culture bottles with monobacterial growth were included in the study. A 125 µL blood sample taken from blood culture bottles giving positive signals was plated on Müller-Hinton agar (Condalap, Spain) in 9 cm petri dishes, and antibiotic discs according to EUCAST recommendations for each bacterium were placed on top. The susceptibility of the microorganisms was measured and recorded after 4, 6, and 8 hours according to EUCAST recommendations.

The recorded antimicrobial susceptibility results were compared with the results recorded in the standard disc diffusion test and the rates of categorical agreement (CA-same clinical category), minor error (mE-reporting a moderately susceptible result as susceptible/resistant), major error (ME-reporting a result that should be susceptible as resistant), very major error (VME-reporting a result that should be resistant as susceptible) and area of technical uncertainty (ATU) were recorded (8,9).

In the study, data were given as numbers and percentages.

RESULTS

A total of 103 bacterial isolates including 19 *S. aureus*, 10 *Enterococcus spp*., 24 *Escherichia coli*, 24 *Klebsiella pneumoniae*, 13 *Pseudomonas aeruginosa*, and 13 *Acinetobacter baumannii* were included in the study. When the RAST method was compared with the standard disc diffusion method, no major errors were detected in *E. coli* isolates and 100% agreement between the methods was found for all hours evaluated against imipenem, meropenem, gentamicin, and trimethoprim-sulfamethoxazole (Table 1). Similarly, a full agreement was found for *K. pneumoniae* isolates against meropenem at hours 6 and 8 (Table 2). The highest error rate was observed for tobramycin in *E. coli* isolates and imipenem in *K. pneumoniae* isolates.

For *S. aureus* isolates included in the study, minor errors in RAST results were not observed for any antibiotic, whereas VMEs were found for all antibiotics (Table 3).

For *Enterococcus spp*. isolates, a full inter-method agreement was found for gentamicin and linezolid, but the vancomycin result was identified as an ATU for all isolates tested (Table 4).

For *A. baumannii* isolates, no minor error was detected for any antibiotic, whereas 100% inter-method agreement was found for imipenem, meropenem, ciprofloxacin, levofloxacin and gentamicin (Table 5).

According to the results of the RAST method, VMEs, and ATUs were detected in *P. aeruginosa* isolates against many antibiotics. Minor errors were found only against amikacin (Table 6).

DISCUSSION

There are several studies based on direct inoculation from positive blood culture bottles to reduce the reporting time of bloodstream infections. Setting appropriate cut-off values is a prerequisite for the correct interpretation of early results. EUCAST has published guidelines on this topic. Many studies show that the RAST test is promising in this regard, although it detects erroneous findings (10,11).

In our study, the number of samples with growth at 4, 6, and 8 hours and the susceptibility patterns were investigated using the RAST method for the antibiotics and bacteria recommended by EUCAST. For all strains included in the study, it was observed that the number of samples with growth and evaluated samples, especially at 4 and 6 hours, was less than the number of samples processed, and the number of samples that could be evaluated increased with increasing incubation time. This situation was accepted as a natural consequence of bacteriological culture but was considered to be a limiting situation in studies to be performed with the RAST method.

In a study comparing the RAST method and the standard disc diffusion method in *E. coli* isolates the categorical agreement rate between the two tests was found as <90% for piperacillin-tazobactam, levofloxacin, and tobramycin, whereas it was found as ≥90% for all other antibiotics (9). In our study, the inter-method agreement was found to be 100% for imipenem, meropenem, gentamicin, and trimethoprim-sulfamethoxazole in *E. coli* isolates. When the same comparison was made for *K. pneumoniae* isolates, the agreement rate was ≥90% in all time periods for cefotaxime, ceftazidime, meropenem, gentamicin, and trimethoprim-sulfamethoxazole. For *K. pneumoniae* isolates, the concordance rates for imipenem were 62.5%,

Table 1. Comparison of RAST and disc diffusion methods in *E. coli* isolates (n=24)

Antibiotics / Hours	4 hours	6 hours	8 hours
Piperacillin-tazobactam			
Number of growth	17	22	24
CA, n (%)	11 (64.7)	18 (81.8)	21 (87.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	2 (11.8)	1 (4.5)	1 (4.2)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	4 (23.5)	3 (13.6)	2 (8.3)
Cefotaxime			
Number of growth	15	19	21
CA, n (%)	14 (93.3)	18 (94.7)	20 (95.2)
mE, n (%)	1 (6.7)	1 (5.3)	1 (4.8)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime			
Number of growth	15	22	24
CA, n (%)	14 (93.3)	21 (95.5)	23 (95.8)
mE, n (%)	1 (6.7)	1 (4.5)	1 (4.2)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem			
Number of growth	16	17	17
CA, n (%)	16 (100)	17 (100)	17 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem			
Number of growth	17	22	24
CA, n (%)	17 (100)	22 (100)	24 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ciprofloxacin			
Number of growth	16	21	24
CA, n (%)	15 (93.8)	19 (90.5)	22 (91.7)
mE, n (%)	0 (0.0)	1 (4.8)	1 (4.2)
ME, n (%)	1 (6.3)	1 (4.8)	1 (4.2)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Levofloxacin			
Number of growth	16	18	18
CA, n (%)	15 (93.8)	16 (88.9)	16 (88.9)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	1 (6.3)	1 (5.6)	1 (5.6)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	1 (5.6)	1 (5.6)
Amikacin			
Number of growth	16	22	24
CA, n (%)	16 (100)	22 (100)	22 (91.7)
mE, n (%)	0 (0.0)	0 (0.0)	2 (8.3)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 2. Comparison of RAST and disc diffusion methods in *K. pneumoniae* isolates (n=24)

Antibiotics / Hours	4 hours	6 hours	8 hours
Piperacillin-tazobactam			
Number of growth	17	22	24
CA, n (%)	15 (88.2)	19 (86.4)	21 (87.5)
mE, n (%)	0 (0.0)	1 (4.5)	1 (4.2)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	2 (11.8)	2 (9.1)	2 (8.3)
Cefotaxime			
Number of growth	14	20	22
CA, n (%)	13 (92.9)	18 (90.0)	20 (90.9)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	1 (7.1)	2 (10.0)	2 (9.1)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime			
Number of growth	14	22	22
CA, n (%)	14 (100)	20 (90.9)	20 (90.9)
mE, n (%)	0 (0.0)	1 (4.5)	1 (4.5)
ME, n (%)	0 (0.0)	1 (4.5)	1 (4.5)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem			
Number of growth	16	19	20
CA, n (%)	10 (62.5)	13 (68.4)	14 (70.0)
mE, n (%)	1 (6.3)	1 (5.3)	1 (5.0)
ME, n (%)	1 (6.3)	1 (5.3)	1 (5.0)
VME, n (%)	4 (25.0)	4 (21.1)	3 (15.0)
ATU, n (%)	0 (0.0)	0 (0.0)	1 (5.0)
Meropenem			
Number of growth	17	23	24
CA, n (%)	16 (94.1)	23 (100)	24 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (5.9)	0 (0.0)	0 (0.0)
Ciprofloxacin			
Number of growth	17	23	24
CA, n (%)	15 (88.2)	21 (91.3)	23 (95.8)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	2 (11.8)	2 (8.7)	1 (4.2)
Levofloxacin			
Number of growth	16	19	19
CA, n (%)	15 (93.8)	17 (89.5)	18 (94.7)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (6.3)	2 (10.5)	1 (5.3)
Amikacin			
Number of growth	16	22	24
CA, n (%)	14 (87.5)	20 (90.9)	22 (91.7)
mE, n (%)	1 (6.3)	1 (4.6)	1 (4.2)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	1 (6.3)	1 (4.6)	1 (4.2)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 1. Comparison of RAST and disc diffusion methods in *E. coli* isolates (n=24) *continued*

Gentamicin			
Number of growth	13	18	20
CA, n (%)	13 (100)	18 (100)	20 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Tobramycin			
Number of growth	16	19	21
CA, n (%)	14 (87.5)	16 (84.2)	17 (80.9)
mE, n (%)	0 (0.0)	0 (0.0)	1 (4.8)
ME, n (%)	2 (12.5)	3 (15.8)	2 (9.5)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	1 (4.8)
Trimethoprim-sulfamethoxazole			
Number of growth	16	20	20
CA, n (%)	16 (100)	20 (100)	20 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 2. Comparison of RAST and disc diffusion methods in *K. pneumoniae* isolates (n=24) *continued*

Gentamicin			
Number of growth	15	21	23
CA, n (%)	14 (93.3)	20 (95.2)	21 (91.3)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	1 (6.7)	1 (4.8)	1 (4.3)
ATU, n (%)	0 (0.0)	0 (0.0)	1 (4.3)
Tobramycin			
Number of growth	16	21	23
CA, n (%)	15 (93.8)	16 (76.2)	18 (78.3)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (6.3)	5 (23.8)	5 (21.7)
Trimethoprim-sulfamethoxazole			
Number of growth	16	19	20
CA, n (%)	15 (93.8)	18 (94.7)	19 (95.0)
mE, n (%)	1 (6.3)	1 (5.3)	1 (5.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 3. Comparison of RAST and disc diffusion methods in *S. aureus* isolates (n=19)

Antibiotics / Hours	**4 hours**	**6 hours**	**8 hours**
Cefoxitin			
Number of growth	6	9	19
CA, n (%)	6 (100)	9 (100)	17 (89.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	2 (10.5)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Clindamycin			
Number of growth	5	9	19
CA, n (%)	5 (100)	8 (88.9)	17 (89.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	1 (11.1)	2 (10.5)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Gentamicin			
Number of growth	4	7	16
CA, n (%)	4 (100)	6 (85.7)	14 (87.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	1 (6.3)
VME, n (%)	0 (0.0)	1 (14.3)	1 (6.3)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Norfloxacin			
Number of growth	5	9	19
CA, n (%)	4 (80.0)	7 (77.8)	16 (84.2)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	1 (20.0)	2 (22.2)	3 (15.8)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 4. Comparison of RAST and disc diffusion methods in *Enterococcus spp*. strains (n=10)

Antibiotics / Hours	**4 hours**	**6 hours**	**8 hours**
Ampicilin			
Number of growth	5	7	10
CA, n (%)	5 (100)	6 (85.7)	10 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	1 (14.3)	0 (0.0)
Gentamicin*			
Number of growth	2	3	7
CA, n (%)	2 (100)	3 (100)	7 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Vancomycin			
Number of growth	-	-	-
CA, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	4 (100)	7 (100)	10 (100)
Linezolid			
Number of growth	2	6	10
CA, n (%)	2 (100)	6 (100)	10 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

68.4%, and 70% at 4, 6, and 8 hours, respectively, whereas these rates were 94.1%, 100%, and 100% for meropenem, respectively.

Erdoğan et al. (12) reported the lowest categorical agreement 91.9% for piperacillin-tazobactam and 92.4% for tobramycin among all antimicrobials tested in their study comparing the RAST method with the standard disc diffusion method. In the same study, it was found that the number of tests concluded at the 4th hour was less than the number of tests concluded at the 6th and 8th hours in *E. coli* and *K. pneumoniae* isolates, and they reported that the EUCAST RAST method is applicable in routine laboratories, can be used to give rapid results with low test cost, but the results should be confirmed by standard methods due to the presence of very large errors. Cao et al. (13) found that the rate of VME in *E. coli* and *K. pneumoniae* isolates was 0.8% in the 4th hour, while no VME was detected in the 6th hour. In the aforementioned study, the advantages of the RAST method such as ease of application and rapid results were emphasized, but it was also reported that further studies were needed.

Martins et al. (14) reported that the majority of zone diameters for *E. coli* and *K. pneumoniae* isolates could be read appropriately after 6 hours of incubation, as highlighted in several studies (15,16). Kansak et al. (17) found that there were more antibiotic and isolate reading errors for *E. coli* and *K. pneumoniae* isolates in the 4th-hour evaluation compared to the 6th- and 8th-hour evaluations, and that the categorical agreement rate increased by 25% for *E. coli* isolates and 50% for *K. pneumoniae* isolates in the 6th-hour evaluation. Only piperacillin-tazobactam had a categorical agreement rate of 84.4% and 88.2% and a minor error rate of 15.6% and 11.8% for *E. coli* and *K. pneumoniae* isolates, respectively. As a result, due to the high minor error rate in the 4th and 6th hours, it was recommended that preliminary reports should be given after the 8th-hour evaluations.

Soo et al. (11) reported that error rates decreased with time in *P. aeruginosa* isolates using the RAST method and that VME was not detected for all antibiotics at the 8th hour, and the authors reported that it would be appropriate to evaluate studies with a large number of isolates. In their study, Kansak et al. (17) found the categorical agreement rate for piperacillin-tazobactam, ceftazidime, and meropenem to be 75% at hour 6 and the categorical agreement rate for all other antibiotics to be ≥90% in *P. aeruginosa* isolates. In the same study, the categorical agreement rate for the antibiotics tested was ≥90% for *A. baumannii* isolates, most of which were multidrug-resistant isolates, and no difference was observed between the 4th and 8th hours. In our study, 92% categorical agreement was found for tobramycin and ciprofloxacin against *P. aeruginosa* isolates at the 8th hour, while the categorical agreement rate was <90% for the other antibiotics at both incubation times. In our study, for *A. baumannii* isolates, the categorical agreement between methods was 37.5% and 70% for amikacin disc at the 4th and 6th hour, and growths detected against sulfamethoxazole at the 4th hour were determined as ATU. Categorical agreement was ≥90% for all other antibiotics and incubation times. The low categorical agreement for *P. aeruginosa* isolates in our study is a remarkable finding and studies with a large number of isolates related to these bacteria are needed. The

Table 5. Comparison of RAST and disc diffusion methods in *A. baumannii* isolates (n=13)

Antibiotics / Hours	4 hours	6 hours	8 hours
Imipenem			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ciprofloxacin			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Levofloxacin			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Amikacin			
Number of growth	8	10	13
CA, n (%)	3 (37.5)	7 (70.0)	11 (84.6)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	1 (10.0)	1 (7.7)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	5 (62.5)	2 (20.0)	1 (7.7)
Gentamicin			
Number of growth	8	9	12
CA, n (%)	8 (100)	9 (100)	12 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Tobramycin			
Number of growth	8	10	13
CA, n (%)	8 (100)	9 (90)	12 (92.3)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	1 (10.0)	1 (7.7)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Trimethoprim-sulfamethoxazole			
Number of growth	8	10	13
CA, n (%)	0 (0.0)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	8 (100)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

values found for *A. baumannii* suggest that the RAST test can be used in routine laboratory applications.

Kansak et al. (17) reported in their study of 20 *S. aureus* isolates that zone diameters were easily assessed at the 4th hour except for two isolates, VME and minor error were not detected, but an 11.1% minor error rate was observed for cefoxitin and gentamicin at the 4th hour. In the aforementioned study, the authors could not detect categorical compliance for vancomycin in *Enterococcus spp*. isolates and VME could not be detected as there were no resistant strains. However, they did detect major errors and VME and therefore characterized the results of vancomycin in *Enterococcus spp*. isolates as categorical non-agreement. Jasuja et al. (9) investigated RAST and Vitek MIC concordance in *S. aureus* isolates and found no VME and minor error for cefoxitin and a BH rate of less than 1%. In the same study, the VME rate for ampicillin in *Enterococcus spp*. isolates was less than 1%, no VME and minor error were detected, only one VME rate (4.2%) was detected for vancomycin, and VME and minor error rates were not reported.

Researchers have reported that the RAST method is rapid and reliable for highly resistant bacteria such as MRSA and VRE (9). In our study, similar to other studies, the categorical agreement rate of cefoxitin susceptibility was ≥90% in all *S. aureus* isolates except for two isolates grown in the 8th hour. Our results suggest that the RAST method can be used in routine laboratories, especially for early detection of MRSA strains and for treatment guidance, but the fact that VME was detected in two isolates of *S. aureus* on cefoxitin disc suggests that studies with larger numbers of isolates are needed and the test should be controlled by the standard disc diffusion method.

In contrast to studies in the literature, in our study, ATU was detected in all incubation times for vancomycin and in only one isolate at the 6th hour for ampicillin in *Enterococcus spp*. isolates and the categorical agreement was 100% for all other antimicrobials. The high level of categorical agreement for *Enterococcus spp*. isolates for antimicrobials other than vancomycin suggest that RAST can be used efficiently in routine laboratory applications.

CONCLUSION

It was concluded that the RAST method is easy to use and does not cause additional work and economic burden in life-threatening infections such as sepsis. The results obtained at the end of the 8th hour suggested that the antibiotics tested by the RAST method could guide the clinician in the use of antibiotics in treatment. However, the results for tobramycin and pipersiline tazobactam for *E. coli* and *K. pneumoniae* isolates, imipenem for *K. pneumoniae* isolates, and norfloxacin for *S. aureus* should be interpreted with caution. For *P. aeruginosa* isolates, susceptibility increased with increasing incubation time for all antibiotics, and for *A. baumannii* isolates, the RAST method gave acceptable and reliable results for all antimicrobials at the end of the 8th hour. Despite the high number of positive results in our data, the fact that compliance rates were low for some antimicrobials supports the idea that such studies should be performed with a larger number of isolates and a larger number of antibiotics.

Table 6. Comparison of RAST and disc diffusion methods in *P. aeruginosa* isolates (n=13)

Antibiotics / Hours	6 hours	8 hours
Piperacillin-tazobactam		
Number of growth	7	12
CA, n (%)	4 (57.1)	9 (75.0)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	3 (25.0)
VME, n (%)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (14.3)	0 (0.0)
Ceftazidime		
Number of growth	7	13
CA, n (%)	4 (57.1)	10 (76.9)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	1 (7.7)
VME, n (%)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (14.3)	2 (15.4)
Imipenem		
Number of growth	7	12
CA, n (%)	4 (57.1)	10 (83.3)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	1 (14.3)	0 (0.0)
VME, n (%)	1 (14.3)	1 (8.3)
ATU, n (%)	1 (14.3)	1 (8.3)
Meropenem		
Number of growth	7	13
CA, n (%)	5 (71.4)	11 (84.6)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	1 (14.3)	0 (0.0)
VME, n (%)	0 (0.0)	1 (7.7)
ATU, n (%)	1 (14.3)	1 (7.7)
Ciprofloxacin		
Number of growth	7	13
CA, n (%)	5 (71.4)	12 (92.3)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	1 (7.7)
ATU, n (%)	2 (28.6)	0 (0.0)
Tobramycin		
Number of growth	7	12
CA, n (%)	6 (85.7)	11 (91.7)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)
VME, n (%)	1 (14.3)	1 (8.3)
ATU, n (%)	0 (0.0)	0 (0.0)
Sefepim		
Number of growth	7	7
CA, n (%)	4 (57.1)	4 (57.1)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	2 (28.6)
VME, n (%)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (14.3)	1 (14.3)
Levofloxacin		
Number of growth	7	7
CA, n (%)	4 (57.1)	4 (57.1)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	2 (28.6)
VME, n (%)	0 (0.0)	1 (14.3)
ATU, n (%)	1 (14.3)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

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Table 6. Comparison of RAST and disc diffusion methods in *P. aeruginosa* isolates (n=13) *continued*

Antibiotics / Hours	6 hours	8 hours
Amikacin		
Number of growth	7	7
CA, n (%)	3 (42.9)	4 (57.1)
mE, n (%)	1 (14.3)	1 (14.3)
ME, n (%)	0 (0.0)	0 (0.0)
VME, n (%)	1 (14.3)	1 (14.3)
ATU, n (%)	2 (28.6)	1 (14.3)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

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Gender Differences in Balance, Lumbar Multifidus Muscle, Pain, and Kinesiophobia in Patients with Lumbar Spinal Stenosis

Lomber Spinal Stenozlu Hastalarda Denge, Lomber Multifidus Kası, Ağrı ve Kinezyofobide Cinsiyet Farklılıkları

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ABSTRACT

Aim: The aim of this study was to examine balance, lumbar multifidus muscle thickness and cross-sectional area (CSA), pain, disability and kinesiophobia levels, and to compare these parameters in terms of gender in patients with lumbar spinal stenosis (LSS).
Material and Methods: This cross-sectional study included 59 patients, 33 (55.9%) female and 26 (44.1%) male, diagnosed with LSS by magnetic resonance imaging (MRI). Low back and leg pains, dynamic and static balances, disability and kinesiophobia levels of patients with LSS were evaluated. Lumbar multifidus muscle thickness and total CSA were obtained from MRI images. Obtained data were compared according to gender.
Results: Females had significantly more low back pain than males (p=0.043), in patients with LSS. Additionally, females with LSS had worse dynamic and static balances (p=0.005, and p=0.001, respectively) and higher levels of disability (p=0.001), and kinesiophobia (p=0.001). Females with LSS had less lumbar multifidus muscle thickness and CSA than males on both the right and left sides. Also, right multifidus muscle thickness correlated with both dynamic (r=-0.289; p=0.027) and static (r=0.349; p=0.007) balances. Significant correlations were detected between low back and leg pain with dynamic and static balances, disability, and kinesiophobia in patients with LSS.
Conclusion: Females with LSS have higher levels of pain, disability, and kinesiophobia than males. Also, LSS affects females' balance functions more and causes further degeneration of the multifidus muscle. Therefore, gender differences should be examined during the clinical follow-up process in LSS.
Keywords: Lumbar spinal stenosis; gender differences; multifidus; balance; pain; kinesiophobia.

ÖZ

Amaç: Bu çalışmanın amacı lomber spinal stenozu (LSS) olan hastalarda denge, lomber multifidus kas kalınlığı ve kesit alanı (cross-sectional area, CSA), ağrı, sakatlık ve kinezyofobi düzeylerini incelemek ve bu parametreleri cinsiyet açısından karşılaştırmaktır.
Gereç ve Yöntemler: Bu kesitsel çalışmaya manyetik rezonans görüntüleme (MRG) ile LSS tanısı konulan 33 (%55,9) kadın ve 26 (%44,1) erkek olmak üzere 59 hasta dahil edildi. LSS'li hastaların bel ve bacak ağrıları, dinamik ve statik dengeleri, özürlülük ve kinezyofobi düzeyleri değerlendirildi. MRG görüntülerinden lomber multifidus kas kalınlığı ve toplam CSA elde edildi. Elde edilen veriler cinsiyete göre karşılaştırıldı.
Bulgular: LSS'li hastalarda, kadınlar erkeklere göre anlamlı olarak daha fazla bel ağrısına sahipti (p=0,043). Ayrıca, LSS'li kadınlarda daha kötü dinamik ve statik denge (sırasıyla p=0,005 ve p=0,001), daha yüksek düzeyde özürlülük (p=0,001) ve kinezyofobi (p=0,001) vardı. LSS'li kadınlarda hem sağ hem de sol tarafta lomber multifidus kas kalınlığı ve CSA erkeklere göre daha azdı. Ayrıca sağ multifidus kas kalınlığı hem dinamik (r=-0,289; p=0,027) hem de statik (r=0,349; p=0.007) denge ile koreleydi. LSS'li hastalarda bel ve bacak ağrısı ile dinamik ve statik dengeler, özürlülük ve kinezyofobi arasında anlamlı korelasyonlar saptandı.
Sonuç: LSS'li kadınlarda erkeklere göre daha yüksek düzeyde ağrı, özürlülük ve kinezyofobi vardır. Ayrıca LSS, kadınların denge fonksiyonlarını daha fazla etkiler ve multifidus kasının daha fazla dejenerasyonuna neden olur. Bu nedenle LSS'de klinik takip sürecinde cinsiyet farklılıkları incelenmelidir.
Anahtar kelimeler: Lomber spinal stenoz; cinsiyet farklılıkları; multifidus; denge; ağrı, kinezyofobi.

INTRODUCTION

Lumbar spinal stenosis (LSS) is a syndrome that occurs when the narrowing in the nerve root canal or intervertebral foramen due to various reasons compresses the neural elements. Pain and neurological symptoms may occur with nerve compression (1). Although seen in both genders, LSS is more common in females and manifests clinically with neurogenic claudication or radicular symptoms (2,3). Patients with LSS exhibit a forward-leaning posture because lumbar extension and walking trigger lower back and leg pain. This posture disorder causes balance problems by causing the person's center of gravity to shift (4,5). In LSS, both static and dynamic balance functions are restricted (6-8).

The multifidus muscle is an important stabilizer of the lumbar spine due to its morphological features such as high cross-sectional area (CSA), dense short muscle fibers, and tonic function of deep fibers. Nerve root compression with spinal stenosis may cause morphological changes in the multifidus muscle (9). Atrophic changes in multifidus muscle morphology are associated with chronic low back pain and increased functional disability in patients with LSS (10,11). In addition, degeneration occurring in the multifidus muscle also causes a decrease in the thickness and CSA of the muscle (12). This condition is associated with loss of balance in various populations (12,13). The results of LSS related to degeneration in paraspinal muscles are contradictory according to gender. Hiyama et al. (14) showed that the multifidus muscle CSA at the lumbar region levels in LSS was less in females. However, Chua et al. (10) determined that the total multifidus CSA did not make a difference between genders. Examining paraspinal muscles, such as multifidus, is important as they play an essential role in maintaining balance and stability.

It is reported that there is a decrease in balance parameters and an increase in pain and disability in patients with LSS (15). Studies in the literature emphasize that the level of disability in females is higher than in males (4,16). This may result from biopsychosocial problems such as decreased tolerance levels in females. However, the evidence on this issue is insufficient (16). Negative beliefs about pain or disease lead to negative reactions in the minds. This leads to kinesiophobia, which is fear-avoidance behavior due to pain experience. Avoidance behavior results in disuse, disability, and depression in individuals and causes individuals to feel more pain. Kinesiophobia is an important factor that may limit function in LSS (17). Although it is stated in the literature that females are more affected by pain, anxiety, and fear (18), there are contradictory results, such as the level of kinesiophobia may be higher in males (19). This situation is not explained in the LSS.

It is observed in the literature that individuals with LSS experience balance problems, an increase in pain and disability levels, and a decrease in paravertebral muscle mass. This situation varies between genders. Additionally, there is insufficient evidence regarding kinesiophobia levels according to gender in LSS. Considering these factors, there are conflicting results in the literature, and it is not clear (10). Examining the differences between genders in patients with LSS is important for choosing the appropriate treatment. Therefore, this study aimed to compare balance, multifidus muscle thickness and CSA, pain, disability, and kinesiophobia levels between genders in patients with LSS and examine their relationship.

MATERIAL AND METHODS

Study Design and Patients

This cross-sectional, descriptive study was conducted in October 2023 and included patients diagnosed with LSS referred to the Neurosurgery outpatient clinic of Karabük University Research and Training Hospital. The patient's LSS was confirmed by a spine specialist using magnetic resonance imaging (MRI). Patients who were diagnosed with LSS at the L4/5 level by MRI were over 18 years old, could stand independently, and volunteered to participate in the study were included in the study. Those who had a history of severe neurological disease (such as Parkinson's, hemiplegia, multiple sclerosis), had a surgical operation on the lumbar region in the last year, had severe joint disease in the lower extremity, malignancy in the spine, had an operation on the lower extremity, and had visual impairment and/or those with vestibular system problems were excluded.

The number of patients to participate in the study was determined based on the multifidus muscle CSA results obtained from a previous study (20). For 80% power, effect size (d) of 0.826, and margin of error (α) of 0.05, at least 24 individuals in both groups were determined as at least 48 with the G*Power v.3.1.9 package.

A total of 59 patients, 33 females, and 26 males, were included in the study. Demographic and clinical characteristics of the patients were recorded. The patients' pain intensity, multifidus muscle thickness and CSA, balance, disability, and kinesiophobia were evaluated and compared according to gender.

The study was approved by the Clinical Research Ethics Committee of Kastamonu University (2023/KAEK-111, 04.10.2023) and was conducted by the Declaration of Helsinki. Written informed consent was obtained from all patients to participate in the study.

Outcome Measures

Pain Intensity: Pain intensity was recorded using the numerical rating scale (NRS) for the waist and leg. NRS ranges from 0 to 10, with a score of 10 indicating the worst pain imaginable (6).

Multifidus Muscle Measurement: Multifidus muscle thickness and muscle CSA, a paraspinal muscle at the L4/5 level, were evaluated using an MRI device (Vision; Siemens Medical Solutions, Erlangen, Germany). The thickness and CSA of the muscle were measured separately on each side. During MRI, patients were placed in a neutral position (supine position, knees extended, and hands on the abdomen). A radiologist evaluated MRI data of lumbar multifidus muscle thickness and CSA. Multifidus muscle thickness from T1 and T2 sagittal and T2 axial images; CSA was evaluated from T2 axial sections with a digital workstation program (Magic View 1000; Siemens, Erlangen, Germany). To determine the CSA of the muscle, it was measured in cm^2, considering the attachment positions to the fascia. The maximum distance between the attachment point of the anterior muscle fascia to the vertebral lamina and the posterior muscle fascia was calculated as the anterior-posterior muscle thickness in

millimeters (21). The reliability of MRI in measuring CSA of the multifidus muscle is acceptable; moderate reliability: 0.858, internal reliability: 0.823 (22).

Balance: The static balance of the individuals was evaluated with the single-leg stance test (SLST). The eyes were open during the test, and the test was performed for 30 seconds (23). Three trials were conducted on the affected side, and the best result obtained was used. Duration was recorded in seconds. The time up and go (TUG) test evaluated individuals' dynamic balance and mobility (24). TUG test measures the patient's backward walking performance in getting up from a standard chair, walking 3 m, turning around, and sitting down again at the starting point (23). The time elapsed during the test was recorded in seconds with a stopwatch.

Disability: The Turkish form of the Oswestry disability index (ODI) was used to evaluate disability. The revised form of ODI, developed to assess functional disability in low back pain, consists of 10 items (pain intensity, personal care, lifting, walking, sitting, standing, sleep, travel, social life, and degree of pain change). Pain-related disability ranges from 0 to 100 points, and higher scores indicate increased disability (25).

Kinesiophobia: The Tampa scale for kinesiophobia (TSK) was used to measure individuals' fear of movement and re-injury. This scale consists of 17 questions, and all items are rated on a 4-point Likert scale (1: strongly disagree to 4: strongly agree). The total score varies from 17 to 68, and as the score increases, the fear of movement increases (17).

Statistical Analysis

IBM SPSS v.22 package was used for statistical data analysis. The normal distribution of the data was evaluated with the Shapiro-Wilk test and histogram graphs. Descriptive statistics were given as mean and standard deviation for normally distributed data and median, interquartile range, minimum, and maximum values for non-normally distributed variables. Categorical data were presented as numbers and percentages. Student's t-test and Mann-Whitney U test were used to compare the two groups, and the chi-square test was used to compare qualitative data. Correlation analysis was used to examine the relationship between quantitative data. Correlation coefficients were considered as >0.89 very strong correlation, 0.70-0.89 strong correlation, 0.40-0.69 medium correlation, and 0.20-0.39 weak correlation (26). Statistical significance was evaluated at $p<0.05$ level.

RESULTS

In total, 35 female and 26 male patients with LSS were screened, and two female patients with LSS were excluded from the study (they did not want to participate in the evaluation due to pain). 33 (55.9%) female LSS patients with a mean age of 58.73±7.93 years and 26 (44.1%) male patients with a mean age of 57.54±8.56 years participated in the study. A comparison of the demographic characteristics of the LSS patients participating in the study according to gender is shown in Table 1.

When the pain results of the groups were compared, low back pain in females with LSS was found to be statistically higher (p=0.043), but leg pain was similar between genders (p=0.089). Multifidus muscle thickness and CSA for both the right and left sides were also less in females with LSS. A comparison of study parameters according to gender is shown in Table 2.

Table 1. Demographic characteristics of the patients

	Female (n=33)	Male (n=26)	p	Total (n=59)
Age (years)	58.73±7.93	57.54±8.56	0.587	58.20±8.16
Height (cm)	162.24±4.84	172.69±4.64	**<0.001**	166.85±7.04
Weight (kg)	70.21±5.80	77.85±6,63	**<0.001**	73.58±7.22
Body mass index (kg/m^2)	26.67±1.96	26.07±1.64	0.218	26.41±1.84
Duration of symptoms (month)	84 (24-120) [15-180]	42 (12-87) [3-240]	0.087	72 (24-120) [3-240]
Affected side, n (%)				
Right	8 (24.2%)	7 (26.9%)		15 (25.4%)
Left	13 (39.4%)	7 (26.9%)	0.591	20 (33.9%)
Bilateral	12 (36.4%)	12 (46.2%)		24 (40.7%)

descriptive statistics were presented in the form of mean±standard deviation, or median (interquartile range, 25th-75th percentile) [minimum-maximum], as appropriate

Table 2. Comparison of pain, muscle parameters, balance, disability, and kinesiophobia according to gender

	Female (n=33)	Male (n=26)	p	Total (n=59)
NRS for low back pain	6.18±1.77	5.15±2.03	**0.043**	5.73±1.94
NRS for leg pain	6.48±1.69	5.69±1.80	0.089	6.14±1.77
Right MF thickness (mm)	38.87±5.29	42.98±6.73	**0.011**	40.68±6.26
Right MF CSA (cm^2)	8.7 (7.0-8.7) [4.5-14.9]	9.3 (8.2-11.6) [6.1-17.4]	**0.001**	8.8 (7.5-10.7) [4.5-17.4]
Left MF thickness (mm)	38.45±5.08	44.10±6.36	**<0.001**	40.94±6.30
Left MF CSA (cm^2)	8.5 (6.9-9.1) [4.9-14.9]	10.1 (8.5-10.5) [6.8-15.9]	**<0.001**	9.0 (7.3-10.1) [4.9-15.9]
TUG test (sec)	13.07±2.72	11.20±2.05	**0.005**	12.25±2.60
SLST (sec)	8.9 (11.2-14.4) [3.3-26.3]	14.8 (9.9-12.6) [3.3-27.2]	**0.001**	10.4 (10.2-14.2) [3.3-27.2]
ODI	66.30±9.76	51.15±12.95	**0.001**	59.63±13.50
TSK	49.70±3.51	45.58±4.02	**0.001**	47.88±4.24

NRS: numeric rating scale, MF: multifidus, CSA: cross-sectional area, TUG: time up and go, SLST: single-leg stance test, ODI: Oswestry disability index, TSK: Tampa scale for kinesiophobia, descriptive statistics were presented in the form of mean±standard deviation, or median (interquartile range, 25th-75th percentile) [minimum-maximum], as appropriate

Table 3. Correlation between pain, muscle parameters, balance, disability and kinesiophobia

		NRS for low back pain	NRS for leg pain	Right MF thickness	Right MF CSA	Left MF thickness	Left MF CSA	TUG test	SLST	ODI
NRS for low back pain	**r**									
	p									
NRS for leg pain	**r**	0.914								
	p	**<0.001**								
Right MF thickness	**r**	-0.260	-0.207							
	p	**0.047**	0.116							
Right MF CSA	**r**	-0.265	-0.207	0.781						
	p	**0.042**	0.115	**<0.001**						
Left MF thickness	**r**	-0.221	-0.177	0.879	0.729					
	p	0.093	0.181	**<0.001**	**<0.001**					
Left MF CSA	**r**	-0.136	-0.101	0.762	0.743	0.815				
	p	0.303	0.448	**<0.001**	**<0.001**	**<0.001**				
TUG test	**r**	0.435	0.380	-0.289	-0.126	-0.212	-0.078			
	p	**0.001**	**0.003**	**0.027**	0.340	0.107	0.558			
SLST	**r**	-0.649	-0.644	0.349	0.207	0.368	0.206	-0.738		
	p	**0.001**	**0.001**	**0.007**	0.116	**0.004**	0.117	**<0.001**		
ODI	**r**	0.590	0.525	-0.309	-0.312	-0.290	-0.227	0.607	-0.713	
	p	**0.001**	**0.001**	**0.017**	**0.016**	**0.026**	0.084	**<0.001**	**<0.001**	
TSK	**r**	0.313	0.274	-0.201	-0.205	-0.186	-0.182	0.627	-0.591	0.781
	p	**0.016**	**0.036**	0.127	0.120	0.157	0.167	**<0.001**	**<0.001**	**<0.001**

NRS: numeric rating scale, MF: multifidus, CSA: cross-sectional area, TUG: time up and go, SLST: single-leg stance test, ODI: Oswestry disability index, TSK: Tampa scale for kinesiophobia

In patients with LSS, weak to moderate correlations were detected between low back and leg pain with TUG, SLST, disability, and kinesiophobia. While right MF muscle thickness correlated with both TUG (r=-0.289; p=0.027) and SLST (r=0.349; p=0.007), the correlation of left MF muscle thickness with SLST was determined (r=0.368; p=0.004). The correlation coefficients between study parameters are shown in Table 3.

DISCUSSION

This study presented that females with LSS had higher levels of low back pain, disability, and kinesiophobia than males. In addition, it was determined that females' balance functions were worse, and their multifidus muscle thickness and CSA were less in patients with LSS. It was determined that increased pain level in patients with LSS was associated with multifidus muscle degeneration, impaired static and dynamic balance, and increased disability and kinesiophobia.

In LSS, chronic low back pain occurs due to degenerative changes in the spine. Pressure on the nerve roots causes pain in the leg that increases with walking (3). Studies have shown that individuals with LSS experience severe pain in the low back and legs (4,11). However, the effect of gender on clinical decision-making in lumbar degenerative diseases has not been adequately examined (10). Although it has been shown in the literature that females have higher pain intensity and more painful symptoms in the general population (27), there are conflicting results between genders in LSS. Kim et al. (4), who investigated symptom severity and pain sensitivity in patients with LSS, showed that, according to VAS, females' low back and leg pains were significantly higher than males. Similarly, it has been reported that low back pain before surgery is more severe in females (28). However, Chua et al. (10) showed that males and females had similar lower back and leg pain levels before surgery in the LSS. In this study, like many studies, it was determined that females experienced more low back pain than males, but leg pain was similar. Due to the different perception levels of pain, females' pain levels may be higher (4). This situation may also be explained by the fact that females are more sensitive to mechanical pain stimuli (28). Therefore, it is important to consider gender differences in the clinical decision-making process.

This study showed that the multifidus muscle CSA at the L4/5 level was reduced more in females with LSS. These findings are like several studies investigating the difference between genders (13,14). Hiyama et al. (14) showed that the multifidus muscle CSA at three different levels in the lumbar region in patients with LSS was less in females. In another study, Chua et al. (10) found that while the total multifidus CSA of males and females was similar in LSS, the functional multifidus CSA was less in females. This difference may be explained by fat infiltration, which affects the muscle's functionality and causes degeneration, is higher in females. In addition, the fact that the patients in Chua et al.'s (10) study were older than this study may affect the results with increased fat infiltration. Also, degenerative changes in the muscle affect functionality by causing a decrease in muscle thickness (12). It is stated that the lumbar multifidus muscle thickness is less in patients with low back pain (21,29). However, the difference that this situation creates between genders is not explained in the LSS. This study determined that females with LSS had less multifidus muscle thickness at the L4/5 level. The change in muscle thickness also leads to a decrease in the muscle's ability to contract (29). This is important in maintaining balance and stability for the multifidus, a deep muscle of the lumbar region (13). In addition, muscle thickness measurement is essential because it provides easy and objective information (21). For this reason, it is necessary to evaluate the morphological features of the muscle for gender during the clinical follow-up process in LSS.

Gender affects balance and physical activity levels. It has been shown that as age increases, females have worse

results than males in both physical and balance performance (30,31). Sung and Ham (31) found that females had worse stability than males during postural changes in patients with LSS. Thornes et al. (6) obtained worse balance results in females with LSS in their study evaluating dynamic balance with the mini-best test. Similarly, in this study, it was determined that both static and dynamic balance were worse in females with LSS. This condition may be associated with pain and muscle degeneration in females. Because it is stated that pain results in claudication, disability, and postural disorders in individuals (7). Also, in LSS, pain intensity, and balance function are negatively related (15). The stability and balance of the lumbar spine depend on the paraspinal muscles surrounding it. Also, muscle atrophies in this region deteriorate the body's stability (32). Similarly, this study found a relationship between balance parameters and muscle thickness, low back, and leg pain. In addition, since the pain intensity and multifidus muscle measurement results of females with LSS differed from males in this study, it may have impacted balance. In this context, it is necessary to evaluate the balance considering these factors regarding gender.

Perceived pain varies according to gender (4). Other factors that cause pain and pathology also cause disability in LSS. Studies emphasize that the level of disability in females with LSS is higher than in males (4,10). Similar to the literature, in this study, females were found to have more disabilities than males according to their ODI scores. Pain perception and tolerance levels appear to be more sensitive in females (4). This may explain the increase in disability among females. Additionally, this study found a relationship between disability with pain and balance. Since pain affects daily activities and causes limitations, previous studies have also presented a relationship between disability and pain in LSS (15,23). In addition, this limitation affects stability, leading to a positive relationship between disability and balance (7,17). This may also explain the difference in balance between the genders. However, the relationship between disability and multifidus muscle CSA has not been adequately examined. Studies conducted in this context have found a relationship between the multifidus and pre-operative disability (10,20). This study found a relationship between multifidus thickness and multifidus CSA and disability. This may be due to the impact of daily activities due to impaired balance and proprioceptive feedback (8) due to multifidus muscle degeneration.

In this study, females' kinesiophobia levels were higher than males. Females are more affected by pain, anxiety, and fear than males (18). However, some studies indicate that the level of kinesiophobia is higher in males (19,33). Rovner et al. (33) found that male kinesiophobia values were higher when the TSK results were examined in a study involving individuals with chronic musculoskeletal pain. However, this situation is unclear in LSS. The greater prevalence of kinesiophobia in males has been associated with higher expectations and fear of losing work capacity and productivity due to re-injury (17,33). Additionally, this study found a relationship between kinesiophobia with pain and disability in LSS. This relationship has been demonstrated in a study at LSS (15). Therefore, the higher prevalence of kinesiophobia in females in this study may be due to decreased expectations due to advanced age and increased pain and disability in females.

CONCLUSION

This study showed that females had higher levels of pain, disability, and kinesiophobia than males. Also, LSS affects females' balance functions more and causes further degeneration of the multifidus muscle. In patients with LSS, increased pain levels are associated with multifidus muscle degeneration, impaired static and dynamic balance, and increased disability and kinesiophobia. Therefore, it is essential to examine these factors specifically for gender in LSS.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Kastamonu University (04.10.2023, 2023-KAEK-111).

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Comparison of Diffusion MRI Findings of High-Graded Primary Brain Tumors and Metastatic Brain Tumors

Yüksek Dereceli Primer Beyin Tümörleri ile Metastatik Beyin Tümörlerinin Difüzyon MR Bulgularının Karşılaştırılması

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ABSTRACT

Aim: Glioblastomas are the highest grade and most mortal primary brain tumors. Cerebral masses that occur with the metastasis of cancers of tissues other than brain are included in the differential diagnosis of glioblastomas. This study aimed to compare the diffusion-weighted imaging signal characteristics of primary and metastatic brain masses and to describe the findings that may be useful in the differential diagnosis.
Material and Methods: Patients with pathologically diagnosed glioblastoma and patients with pathologically diagnosed metastases or radiologically diagnosed brain metastases were included in the study. Diffusion-weighted imaging signal properties in magnetic resonance imaging examinations obtained with a 1.5 Tesla scanner were retrospectively analyzed. The signal features and short and long diameters of the lesions were measured and compared in both patient groups.
Results: A total of 54 patients, 24 glioblastomas, and 30 brain metastases were included in the study. The most common signal feature of diffusion-weighted imaging in the glioblastoma group was heterogeneous hyper- and hypointense areas observed in 20 (83.3%) patients. The most common signal feature in the metastasis group was the peripheral hyperintense ring and central hypointense signal in 16 (53.3%) patients. There was no significant relation found between the number of lesions and the primary brain tumor and metastases.
Conclusion: Although only signal characteristics are used without quantitative assessment in diffusion-weighted imaging, it may be helpful in the differential diagnosis of primary and metastatic brain masses. It is important to remember that the masses in the two groups can have comparable signal properties.
Keywords: Diffusion-weighted imaging; glioblastoma; brain metastasis; signal properties.

ÖZ

Amaç: Glioblastomalar en yüksek dereceli ve en ölümcül primer beyin tümörleridir. Beyin dışı dokulardaki kanserlerin beyne metastazı ile ortaya çıkan beyin kitleleri glioblastomaların ayırıcı tanısında yer almaktadır. Bu çalışmada, primer ve metastatik beyin kitlelerinin difüzyon ağırlıklı görüntüleme sinyal özelliklerinin karşılaştırılması ve ayırıcı tanıda faydalı olabilecek bulguların tanımlanması amaçlandı.
Gereç ve Yöntemler: Çalışmaya patolojik olarak glioblastoma tanısı almış hastalar ile patolojik olarak metastaz tanısı almış veya radyolojik olarak beyin metastazı tanısı almış hastalar dahil edildi. 1,5 Tesla tarayıcı ile elde edilen manyetik rezonans görüntüleme incelemelerindeki difüzyon ağırlıklı görüntüleme sinyal özellikleri geriye dönük olarak analiz edildi. Her iki hasta grubunda lezyonların sinyal özellikleri ile kısa ve uzun çapları ölçüldü ve karşılaştırıldı.
Bulgular: Bu çalışmaya 24 glioblastoma ve 30 beyin metastazı olmak üzere toplam 54 hasta dahil edildi. Glioblastoma grubunda difüzyon ağırlıklı görüntülemenin en yaygın sinyal özelliği 20 (%83,3) heterojen hiper ve hipointens alanlar olarak saptandı. Metastaz grubunda en sık görülen sinyal özelliği 16 (%53,3) hastada periferik hiperintens halka ve santral hipointens sinyal olarak saptandı. Lezyon sayısı ile primer beyin tümörü ve metastazlar arasında anlamlı bir ilişki bulunamadı.
Sonuç: Difüzyon ağırlıklı görüntülemede kantitatif değerlendirme yapılmadan sadece sinyal özellikleri kullanılsa da primer ve metastatik beyin kitlelerinin ayırıcı tanısında yardımcı olabilir. İki gruptaki kitlelerin karşılaştırılabilir sinyal özelliklerine sahip olabileceğini unutmamak önemlidir.
Anahtar kelimeler: Difüzyon ağırlıklı görüntüleme, glioblastom, beyin metastazı, sinyal özellikleri.

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INTRODUCTION

Glial cells are the major source of 75% of adult primary brain cancers. Glioblastomas are the highest grade and most mortal primary brain tumors. Cerebral masses that occur with the metastasis of cancers from non-brain tissues are included in the differential diagnosis of glioblastomas (1,2). Magnetic resonance imaging (MRI) is the primary method for imaging brain masses. Conventional MRI T2 weighted signal features, contrast enhancement, apparent diffusion coefficient (ADC) values, perfusion, and spectroscopy are used to distinguish primary brain tumors from metastases. There are few studies in the literature focusing solely on diffusion-weighted imaging (DWI) signal features. DWI is a method that examines the random movements of water molecules in tissues and is routinely used in the evaluation of cerebral mass (3-5).

We aimed to compare the DWI signal characteristics of primary and metastatic brain masses and to describe the findings that may be useful in the differential diagnosis.

MATERIAL AND METHODS

The study was conducted retrospectively after receiving the approval with decision numbered 2020/15 and dated 04.02.2020 from the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee. Patients who applied to Bolu Abant Izzet Baysal University Training and Research Hospital between 2016 and 2020 and underwent diffusion MRI for various reasons were evaluated. Initially, the number of patients was determined as 72. Patients with a history of previous surgery and intralesional bleeding were excluded from the study. For various reasons, 18 patients were excluded in the study. A total of 54 patients, including 24 glioblastomas and 30 brain metastases, were included in the study. Patients whose primary brain tumors were pathologically confirmed as grade 3 and grade 4 brain tumors by biopsy were included. In the metastasis patient group; patients who were pathologically proven to have metastasis by biopsy or whose non-brain tumor did not have a brain mass at the diagnosis stage and who developed a brain mass during the follow-up interval and were clinically accepted as metastasis were included. In metastatic brain tumors; 17 patients with lung cancer metastasis, 1 patient with malignant melanoma metastasis, 2 patients with epithelial tumor metastasis, 3 patients with renal cell cancer metastasis, 2 patients with colon cancer metastasis, 2 patients with breast cancer metastasis, 1 patient with squamous cell cancer metastasis, 1 patient with stomach cancer metastasis and 1 patient was reported as adenocarcinoma metastasis of unknown primary.

Contrast-enhanced brain MRI and other metastasis screening examinations were performed in the majority of patients included in the study, and due to the purpose of the study, the focus was on DWI. In our clinic, patients referred for contrast-enhanced brain MRI also routinely undergo diffusion MRI, in patients referred from the emergency department with suspicion of stroke, only diffusion MRI examination is performed.

Patients with a previous surgical history or intralesional bleeding were not included in the study. Contrast-enhanced brain MRI images of some patients taken after diagnosis or after surgery were used in the sample images for demonstration purposes. However, when evaluating the lesion signal, only preoperative and diffusion MR images were included.

DWI signal properties in brain MRI examinations were obtained with a 1.5 Tesla MRI (Siemens Magnetom Symphony, Erlangen, Germany) scanner. A 6-channel head coil is used to receive signals. The images were analyzed retrospectively by two radiologists with 4 and 12 years of experience. B1000 images of DWI sequences were evaluated in the DWI examinations of the patients.

Statistical Analysis

Study data were evaluated via IBM SPSS version 23.0. Patients' age, sex, and demographic data were analyzed by descriptive statistical methods. Shapiro-Wilk test was used as the normality test. Student t-test was used to examine the difference between the two groups. Categorical variables were compared with Pearson's chi-square or Fisher's exact test. The statistical significance level of $p<0.05$ was considered significant.

RESULTS

A total of 54 patients, 24 glioblastomas, and 30 brain metastases were included in the study. No significant difference was found between the groups both in age and gender. Details of the demographic characteristics of the patients were given in Table 1. The most common signal feature in the glioblastoma group was heterogeneous hyper- and hypointense areas in 20 patients. The most common signal feature in the metastasis group was the peripheral hyperintense ring and central hypointense signal in 16 patients. A detailed description of the signal characteristics was presented in Table 2. Heterogeneous hyperintense and hypointense areas were significantly more common in high-grade brain tumors. Peripheral hyperintense ring and central hypointense signal appearance were significantly more common in the metastasis group. Homogeneous hypointense signal appearance was also observed significantly more in the metastasis group. Patients with solitary and multiple masses were included in the study. In patients with multiple lesions signal characteristics of those lesions did not show different features in both primary tumor and metastasis groups.

The short and long diameters of the lesions were measured and compared in both patient groups. The length of the short diameters in the glioblastoma group was significantly higher than the metastasis group (31.50±9.25 vs. 23.46±11.55, p=0.030). Although the longest diameters were larger in the glioblastoma group, there was no significant difference between the groups (p=0.068). The relationship between the number of lesions seen in the patient groups and the diagnosis of patients was evaluated.

Table 1. Demographics of the patients

	HG Tumors (n=24)	**Metastasis (n=30)**	**p**
Age (years), mean±SD	62.14±3.39	61.91±1.61	0.829
Gender, n (%)			
Male	12 (50.0)	23 (76.7)	0.079
Female	12 (50.0)	7 (23.3)	

HG: high-grade, SD: standard deviation

Table 2. Signal characteristics of the patients

	HG Tumors (n=24)	Metastasis (n=30)	p
Signal appearance in the diffusion-weighted examination, n (%)			
Heterogeneous hyperintense and hypointense areas (Figure 1)	20 (83.3)	5 (16.7)	**<0.001**
Peripheral hyperintense ring and central hypointense (Figure 2)	2 (8.3)	16 (53.3)	
Homogeneous hyperintense (Figure 3)	2 (8.3)	4 (13.3)	
Homogeneous hypointense (Figure 4)	0 (0.0)	5 (16.7)	

HG: high-grade

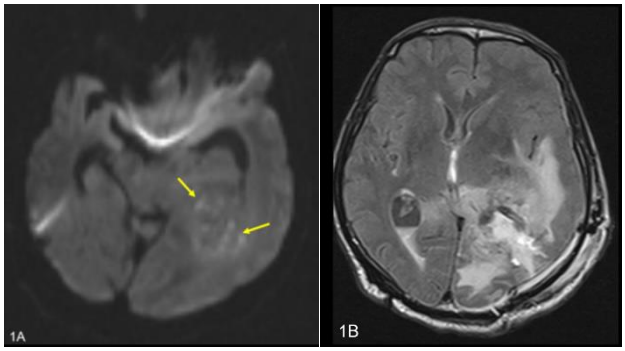

Figure 1. A) Diffusion-weighted imaging b1000, mass in the left parietotemporal lobe of a 73-year-old male patient with heterogeneous hyperintense and hypointense areas (arrows), and **B)** contrast-enhanced T1 weighted image, pathology: glioblastoma

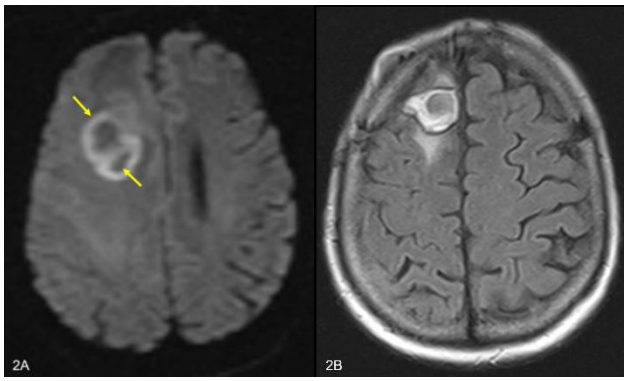

Figure 2. A) A 72-year-old male patient with peripheral hyperintense ring, and central hypointense lesion on the right frontal lobe anterior diffusion-weighted images (arrows), and **B)** contrast-enhanced T1 weighted image, pathology: lung cancer metastasis

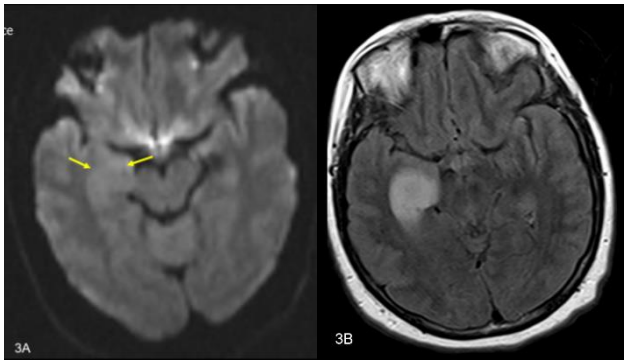

Figure 3. A) A 45-year-old female patient with a homogeneous hyperintense mass in the parahippocampal gyrus in the right medial temporal lobe on the diffusion-weighted image (arrows), and **B)** T2-weighted sequence image, pathology: glioblastoma

There were 18 solitary and 6 multiple masses in the glioblastoma group, 16 solitary and 14 multiple in the metastasis group, and no significant difference was found between the groups (p=0.072).

DISCUSSION

DWI is useful in the differential diagnosis of primary and metastatic cerebral masses, although only signal features are used without quantitative measurement (6,7). It has been proposed that a decrease in ADC values during imaging corresponds to increased cellularity, which may help determine whether tumor cells have invaded the surrounding tissues. This idea has been supported by several studies that compared the peritumoral edema of high-grade gliomas with metastases (8-14).

The most common signal we encountered in primary brain tumors was observed as heterogeneous hyperintense and hypointense signals on DWI. Since the tumor cell load is high accompanied by necrotic areas in primary glial tumors, they appear as hyper- and hypointense areas (15). The peripheral hyperintense ring was observed much more frequently in the metastasis group. This signal feature was seen in a total of 18 patients, 16 of whom were detected in the metastasis group, and was observed in approximately 89% of the patients in the metastasis group. Central hypointense areas represent necrotic areas. We think that a peripheral ring is formed because there is cellular density in the peripheral areas. Homogeneous hyperintense signal feature was seen in both groups and were not common in both groups. We think that this signal feature is seen in non-necrotic tumors. Since there are small numbers in both groups, it is not reliable in terms of discrimination. Homogeneous hypointense signal was observed only in the metastasis group and in a small number of patients. We

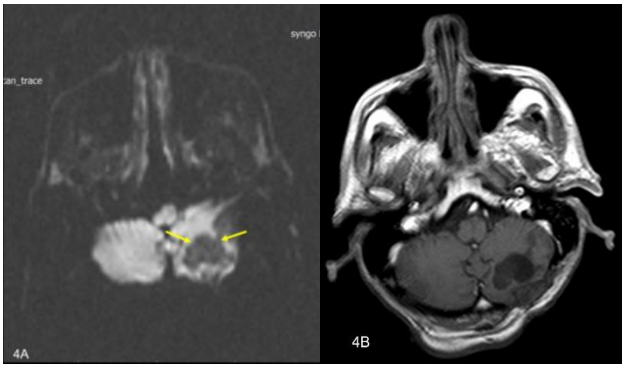

Figure 4. A) A 69-year-old male patient with homogeneous hypointense lesion in the left cerebellar hemisphere on the diffusion-weighted image, and **B)** contrast-enhanced T1 weighted image, pathology: metastatic colon cancer

think that this signaling feature is due to tumors that are necrotic in the center and have low cell density in the periphery.

Even though the primary cancer of the patients in the metastasis group was the same, we detected different diffusion signals in patients with different subtypes. For example, in patients with lung cancer metastases, the signal features of small cell lung cancer metastases and non-small cell lung cancer metastases were not always consistent. While a homogeneous hyperintense signal was observed in some of the patients with non-small cell lung cancer metastases, a peripheral hyperintense ring and central hypointense were observed in small cell lung cancer metastases.

There was no difference between glioblastoma and metastasis groups, whether the cerebral masses were solitary or multiple. Although the long diameters of the masses were found to be large in the metastasis and the short diameters of the masses in the glioblastoma group, more patients and contrast-enhanced examinations are required for the appropriate evaluation of these mass sizes. Due to our small number of patients, subtype research could not be performed in the primary and metastasis patient groups. The fact that our study was single-center and had a small number of patients was an important limitation.

CONCLUSION

Diffusion-weighted imaging may be useful in the differential diagnosis of primary and metastatic cerebral masses, although only signal features are used without quantitative measurement. Our findings may be useful in daily routine radiology practice to differentiate primary and metastatic cerebral masses without the use of further investigations.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University (04.02.2020, 2020/15).

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Increased Nuchal Translucency and Pregnancy Outcomes: A Tertiary Center Data

Artmış Nukal Translusensi ve Gebelik Sonuçları: Bir Üçüncü Basamak Merkez Verileri

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ABSTRACT

Aim: This study aimed to evaluate the pregnancy outcomes of patients who applied to our clinic between the 11th and 14th weeks of pregnancy and whose nuchal translucency (NT) measurement was ≥1.5 multiples of the median (MoM).
Material and Methods: The study included 85 patients whose NT measurement was determined ≥1.5 MoM and pregnancy results were available. Demographic characteristics of the patients, prenatal invasive diagnostic test results, fetal anomaly screening, fetal echocardiography (ECHO) results, and neonatal and obstetric results were evaluated.
Results: Abnormal karyotype was detected in 10.6% (n=9) of the patients. Trisomy 21 was the most common chromosomal anomaly. Fetal structural anomaly was detected in 29.4% (n=25) of the patients. A structural fetal anomaly was detected in 21% (n=13) of fetuses with normal karyotypes and 66.7% (n=6) of fetuses with abnormal karyotypes. Cardiac anomalies were found to be the most common anomalies with 9.7% (n=6) in patients with normal karyotype. NT and NT MoM values in patients with fetal structural (both p=0.001) or chromosomal anomalies (p=0.011, and p=0.019, respectively) were found significantly higher than those without. NT and NT MoM values in patients whose pregnancies resulted in fetal loss were found significantly higher than in patients who had a live birth (both p=0.001).
Conclusion: Increasing NT or NT MoM values indicate an increase in the risk of chromosomal anomalies, structural anomalies, and poor pregnancy outcomes in the fetus. Fetal anomaly screening and fetal ECHO should be recommended in patients with increased NT, even if a normal karyotype is detected.
Keywords: Abnormal karyotype; anomaly; increased nuchal translucency.

ÖZ

Amaç: Bu çalışmanın amacı, gebeliğin 11. ile 14. haftaları arasında kliniğimize başvuran ve nukal translusensi (NT) ölçümleri ortancanın ≥1,5 katı (multiples of the median, MoM) olan hastaların gebelik sonuçlarını değerlendirmektir.
Gereç ve Yöntemler: Çalışmaya NT ölçümü ≥1,5 MoM olarak tespit edilen ve gebelik sonuçlarına ulaşılabilen 85 hasta dahil edildi. Hastaların demografik özellikleri, prenatal invaziv tanı testi sonuçları, fetal anomali taraması, fetal ekokardiyografi (EKO) sonuçları ile neonatal ve obstetrik sonuçları değerlendirildi.
Bulgular: Hastaların %10,6 (n=9)'sında anormal karyotip saptandı. Trizomi 21 en sık görülen kromozom anomalisiydi. Hastaların %29,4 (n=25)'ünde fetal yapısal anomali tespit edildi. Normal karyotipli fetusların %21 (n=13)'inde ve anormal karyotipli fetusların %66,7 (n=6)'sinde yapısal fetal anomali tespit edildi. Kardiyak anomaliler normal karyotipli hastalarda %9,7 (n=6) ile en sık görülen anomali olarak bulundu. NT ve NT MoM değerleri, fetal yapısal (her iki p=0,001) veya kromozomal anomalisi olan hastalarda (sırasıyla p=0,011 ve p=0,019) olmayanlara göre anlamlı olarak daha yüksek bulundu. Gebeliği fetal kayıp ile sonuçlanan hastalarda NT ve NT MoM değerleri, canlı doğum yapan hastalara göre anlamlı olarak daha yüksek bulundu (her iki p=0.001).
Sonuç: NT veya NT MoM değerlerinin artması fetusta kromozomal anomaliler, yapısal anomaliler görülme riskinin ve olumsuz gebelik sonuçlarının artmasına işaret eder. NT artışı olan hastalarda normal karyotip tespit edilse bile fetal anomali taraması ve fetal EKO önerilmelidir.
Anahtar kelimeler: Anormal karyotip; anomali; artmış nukal translusensi.

INTRODUCTION

Detecting fetal anomalies in the early stages of pregnancy is one of the most important purposes of prenatal sonography. Nuchal translucency (NT) occurs due to fluid accumulation in the subcutaneous tissue at the back of the fetal neck and can be evaluated by ultrasonography (USG) between the 10^{th} and 14^{th} weeks of pregnancy. NT measurement is the most important part of the first trimester combined screening test for aneuploidy screening (1). By combining NT measurement with maternal serum markers PAPP-A and free β-hCG, Down syndrome detection rates reach 80-90% (2-4).

Increased NT is defined as the measured NT value being ≥95^{th} percentile or ≥1.5 multiples of the median (MoM) according to the fetal crown-rump length (CRL) (5). In addition to leading to the detection of chromosomal diseases, NT increase is also associated with poor pregnancy outcomes such as many genetic syndromes, fetal structural anomalies, and fetal loss (6-11).

In this study, it was aimed to evaluate the prenatal and postnatal outcomes of pregnant women who applied to our clinic between the 11^{th} and 14^{th} weeks of gestation and whose NT measurement was ≥1.5 MoM.

MATERIAL AND METHODS

Approval for the study was received from the local ethics committee of Van Yüzüncü Yıl University (approval date: 18.11.2022 and number: 11-27). The outpatient records and electronic hospital information system of 18,505 patients who applied to Van Yüzüncü Yıl University Faculty of Medicine Perinatology clinic between 2018 and 2022 were retrospectively scanned. Patients with NT measurement ≥95^{th} percentile between the 11^{th} and 14^{th} weeks of gestation were included in the study. CRL and NT measurements were performed in accordance with the measurement criteria determined by the Fetal Medicine Foundation (FMF) (12). NT measurements were converted to MoM values according to CRL with FMF's NT calculation programs. 105 patients with increased NT measurements were identified. However, 20 patients were excluded from the study because their pregnancy results could not be obtained. All patients were evaluated at the perinatology council and families were given detailed information about the prognosis. For prenatal diagnosis, patients were offered the option of invasive diagnostic tests, chorionic villus sampling, and amniocentesis. Quantitative fluorescent polymerase chain reaction (QF-PCR) and cytogenetic culture were requested from all patients who had an invasive diagnostic test, according to the recommendations of the genetics department. Fetal anomaly screening was performed between the 18^{th} and 22^{nd} weeks of gestation. Voluson E6 model (General Electric Healthcare, USA) 2-5 MHz transabdominal probe was used for NT evaluation and fetal anomaly screening. By scanning the digital hospital data system, the demographic characteristics of the patients, prenatal karyotype results if performed, fetal anomaly screening, and fetal ECHO results were found. By scanning the hospital data system, the pregnancy results of the patients who continued their pregnancy follow-up in our hospital were obtained. All patients included in the study were contacted via their registered telephone information and asked how the pregnancy ended (spontaneous abortion, intrauterine fetal death, termination, live birth) and whether any abnormalities (mental, motor development, organ-system dysfunction) were detected during the postnatal neonatal examination and the postnatal follow-up of the child.

Statistical Analysis

Shapiro-Wilk test, and skewness and kurtosis values were used to check whether the continuous measurements in the study were normally distributed. Parametric tests were applied for normally distributed variables. Descriptive statistics were expressed as mean±standard deviation, median, interquartile range, minimum-maximum, number of patients, and percentage. Independent samples t-test was used to compare measurements between the groups. The statistical significance level was taken as $p<0.05$, and the IBM SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for analyses.

RESULTS

In the present study, 18,505 patient data were examined retrospectively. 105 (0.6%) patients with NT measurement ≥95^{th} percentile according to CRL were identified. However, 20 patients were excluded from the study because their pregnancy results could not be obtained.

The median age of patients was 29 (range, 19-45) years, the median number of gravida was 2 (range, 1-12), parity was 1 (range, 0-5), abortions was 0 (range, 0-6), living children was 1 (range, 0-5), the median gestational week was 12^{+4} (range, 11^{+0}-14^{+2}) in the form of week^{+day}. The median CRL was 56 (range, 41-83) mm, the median NT was 3.8 (range, 3.0-7.9) mm and the median NT MoM was 2.6 (range, 1.6-6.4). Demographic and ultrasonographic characteristics of the patients were shown in Table 1.

Of the patients, 31.8% (n=27) wanted to have a prenatal invasive diagnostic test for karyotype analysis, and chorionic villus sampling was performed in 10 patients, and amniocentesis was performed in 17 patients. A chromosomal anomaly was found in 29.6% (n=8) of the patients who had invasive diagnostic testing. While 62.5% (n=5) of the chromosomal anomalies were Trisomy 21, 25% (n=2) were Trisomy 18, and 12.5% (n=1) were 45XO. Fetal ECHO was performed in 30.6% (n=26) of the patients. Fetal cardiac anomaly was detected in 34.6% (n=9) of the patients who underwent fetal ECHO. Fetal anomaly screening was performed in 75.3% (n=64) of the patients.

Table 1. Demographic and ultrasonographic characteristics of the patients

	Median	IQR	Min-Max
Age (years)	29	25-34	19-45
Gravida	2	2-4	1-12
Parity	1	0.5-2	0-5
Abortion	0	0-1	0-6
Living child	1	0.5-2	0-5
Pregnancy week	12^{+4}	12^{+0}-13^{+4}	11^{+0}-14^{+2}
CRL (mm)	56	52-70	41-83
NT (mm)	3.8	3.2-4.8	3.0-7.9
NT MoM	2.6	2.1-3.6	1.6-6.4

CRL: crown-rump length, NT: nuchal translucency, MoM: multiples of the median, mm: millimeter, IQR: interquartile range (25^{th}-75^{th} percentile)

Congenital fetal anomaly was detected in 29.7% (n=19) of the patients who underwent fetal anomaly screening. 74.1% (n=63) of the patients' pregnancies resulted in live births, 11.8% (n=10) in intrauterine fetal death, 5.9% (n=5) in spontaneous abortion, and 8.2% (n=7) in termination. During the examination and follow-up of the newborn after live birth, normal findings were detected in 79.3% (n=50), and abnormal findings were found in 15.9% (n=10). Three babies died after live birth. Prenatal and postnatal results of the patients were shown in Table 2.

When prenatal and postnatal results were evaluated together, the chromosomal anomaly was found in 10.6% (n=9) of the patients. Fetal karyotype could not be determined in 16.5% (n=14) of the patients. Fetal karyotypes could not be determined in 11 patients because their pregnancies resulted in fetal loss without invasive diagnostic testing after the detection of NT increase, and in three patients with neonatal death after live birth, they did not have karyotype analysis in both the prenatal and postnatal periods. Normal karyotype was detected in 72.9% (n=62) of the patients, while fetal anomaly was detected in 29.4% (n=25). A structural fetal anomaly was detected in 21% (n=13) of the fetuses with normal karyotypes, 66.7% (n=6) of fetuses with abnormal karyotypes, and 42.9% (n=6) of fetuses with unknown karyotypes. While the live birth rate was found to be 76.9% (n=10) in fetuses with normal karyotypes with anomalies, this rate was found to be 98% (n=48) in those without anomalies. The results of the patients according to their chromosomal anomaly status were shown in Table 3.

Of the patients with prenatal fetal structural anomaly, 47.3% (n=9) had cardiac anomaly and 26.3% (n=5) had hydrops fetalis. Central nervous system anomaly was detected in four patients, facial anomaly in two patients, kidney anomaly in two patients, anterior abdominal wall defect in two patients, and extremity defect in two patients. 47.3% (n=9) of the anomalies were multiple anomalies. When prenatal and postnatal results were evaluated together, the most common anomalies were cardiac anomalies at 9.7% (n=6) and facial anomalies at 4.8% (n=3) in patients with normal karyotypes. Among the patients with abnormal karyotype detected prenatally, only the pregnancy of the patient whose result was 45XO resulted in live birth, while the pregnancy of four patients was terminated upon their request and the pregnancies of three patients resulted in intrauterine fetal death. The characteristics and pregnancy outcomes of patients with karyotype and/or fetal anomalies were shown in Table 4.

During the examination and follow-up of the newborn after live birth, abnormal findings were detected in 20.6% (n=13) of the babies. New findings were detected in the postnatal period in seven of the patients who did not have prenatal invasive diagnostic testing and fetal anomaly screening. During the newborn examination and follow-up after live birth, cleft lip in two babies, cleft palate and undescended testicle in one baby, ventricular septal defect (VSD) and aortic coarctation in one baby, VSD in one baby, Trisomy 21 in one baby, and growth retardation in one baby were detected during follow-up. Anomalies detected in the newborn examination and follow-up after live birth in patients who did not have a prenatal diagnostic test and fetal anomaly screening were shown in Table 5.

NT measurements and NT MoM values of patients with abnormal karyotypes were found to be significantly higher than those of patients with normal karyotypes (p=0.011, and p=0.019, respectively). NT measurements and NT MoM values of patients with fetal structural anomaly were found to be significantly higher than those of patients without fetal structural anomaly (both p=0.001). NT measurements and NT MoM values of patients whose pregnancies resulted in fetal loss were significantly higher than those of patients who had a live birth (both p=0.001). During the examination and follow-up of the newborn after live birth, NT measurements and NT MoM values of patients with pathological findings were found to be significantly higher than those of patients with normal findings (both p=0.001). The comparison of prenatal and postnatal results in terms of NT and NT MoM values was shown in Table 6.

Table 2. Prenatal and postnatal outcomes of the patients

	n (%)
Invasive diagnostic testing	
Normal karyotype	19 (22.4)
Abnormal karyotype	8 (9.4)
Did not do	58 (68.2)
Fetal echocardiography	
Normal	17 (20.0)
Anomaly detected	9 (10.6)
Did not do	59 (69.4)
Fetal anomaly screening	
Normal	45 (52.9)
Anomaly detected	19 (22.4)
Did not do	21 (24.7)
Pregnancy outcome	
Live birth	63 (74.1)
Intrauterine fetal death	10 (11.8)
Spontaneous abortion	5 (5.9)
Termination	7 (8.2)
Status of the newborn after live birth	
Normal	50 (79.3)
Abnormal	10 (15.9)
Ex	3 (4.8)

Table 3. Results of patients according to chromosomal anomaly status

	Karyotype						
	Normal (n=62)		**Abnormal (n=9)**			**Unknown (n=14)**	
	Anomaly (+) (n=13, 21.0%)	**Anomaly (-) (n=49, 79.0%)**	**Anomaly (+) (n=6, 66.7%)**	**Anomaly (-) (n=2, 22.2%)**	**Unknown (n=1, 11.1%)**	**Anomaly (+) (n=6, 42.9%)**	**Unknown (n=8, 57.1%)**
Live birth	10 (76.9)	48 (98.0)	0 (0.0)	2 (100)	0 (0.0)	3 (50.0)	0 (0.0)
Intrauterine ex	0 (0.0)	1 (2.0)	3 (50.0)	0 (0.0)	0 (0.0)	2 (33.3)	4 (50.0)
Abortion	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	4 (50.0)
Termination	3 (23.1)	0 (0.0)	3 (50.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)

Table 4. Characteristics and pregnancy outcomes of patients with karyotype and/or fetal structural anomalies

NT (mm)	NT MoM	Karyotype result	Fetal anomaly scan result	Pregnancy result
6.6	3.8	Did not do	Omphalocele + hypoplastic left heart	IU fetal death
5.2	3.6	Trisomy 21	AVSD + spina bifida	IU fetal death
4.4	3.2	Normal	Aortic stenosis	Live birth
4.5	4.2	Did not do	VSD	Live birth
6.8	5.1	Trisomy 21	Hydrops fetalis	IU fetal death
7.7	4.3	Trisomy 21	Did not do	Termination
4.8	3.8	Normal	Hydrocephalus	Termination
5.6	5.3	Trisomy 18	Hydrops fetalis	IU fetal death
4.8	3.4	Did not do	VSD + aortic coarctation + multicystic kidneys	Live birth newborn ex
4.8	3.5	Normal	Hypoplastic left heart	Termination
4.2	3.2	Trisomy 18	Diaphragmatic hernia + bilateral club foot + hydrops fetalis	Termination
4.9	3.4	Did not do	Pleural effusion + micrognathia + hypotelorism	Live birth newborn ex
7.0	5.3	Normal	Omphalocele + multicystic kidney	IU fetal death
7.0	3.8	Normal	Dandy walker variant + diaphragmatic hernia + bilateral club foot	Termination
7.0	5.3	Did not do	Hydrops fetalis	IU fetal death
3.8	3.3	Did not do	Micrognotia + cerebellar hypoplasia + nasal hypoplasia	Live birth newborn ex
7.5	6.4	Did not do	Truncus arteriosus	Spontaneous abortion
4.0	2.9	45XO	No anomalies	Live birth
4.8	2.9	Did not do	VSD	Live birth
6.2	3.2	Trisomy 21	AVSD + nasal hypoplasia	Termination
5.4	4.1	Trisomy 21	Hydrops fetalis	Termination

NT: nuchal translucency, MoM: multiples of the median, AVSD: atrioventricular septal defect, VSD: ventricular septal defect, IU: intrauterine

Table 5. Anomalies detected in newborn examination and follow-up after live birth in patients not had prenatal invasive diagnostic testing and fetal anomaly screening

Anomaly	Number of patients
Cleft palate + undescended testicle	1
Cleft lip	2
Growth failure	1
VSD + Aortic coarctation	1
VSD	1
Trisomy 21	1

VSD: ventricular septal defect

Table 6. Comparison of NT and NT MoM values in terms of prenatal and postnatal results

	Karyotype		p
	Normal	Abnormal	
NT (mm)	4.21±1.23	5.65±1.25	**0.011**
NT MoM	2.86±1.10	3.96±0.90	**0.019**

	Fetal anomaly screening		p
	Normal	Abnormal	
NT (mm)	3.43±0.50	5.46±1.16	**0.001**
NT MoM	2.28±0.64	3.91±0.93	**0.001**

	Pregnancy result		p
	Live birth	Fetal loss	
NT (mm)	3.68±0.66	5.76±1.26	**0.001**
NT MoM	2.46±0.69	4.20±0.86	**0.001**

	Status of the newborn		p
	Normal	Abnormal	
NT (mm)	3.47±0.48	4.51±0.59	**0.001**
NT MoM	2.24±0.49	3.30±0.68	**0.001**

NT: nuchal translucency, MoM: multiples of the median

DISCUSSION

While NT increase allows the detection of chromosomal anomalies, especially Down syndrome, in the prenatal period, it is also associated with poor pregnancy outcomes such as many genetic syndromes, fetal structural anomalies, and fetal loss (6-11). Increased NT is seen in 0.5% to 1.75% of pregnant women in the general population (13,14). During the period when the study was conducted in our clinic, this rate was found to be 0.6% (n=105). Although we are a busy clinic, the reason why we found a rate close to the lower limit stated in the literature is that the majority of patients come to our clinic between the ages of 18-22. We thought it was because they applied for fetal anomaly screening during the gestational weeks.

In our study, only 31.8% (n=27) of the patients had invasive diagnostic testing. When prenatal and postnatal results are evaluated together; Chromosome anomalies were detected in 10.6% of the patients, most commonly Trisomy 21 and Trisomy 18. It has been reported in the literature that chromosomal anomalies are detected in 20% to 44% of the patients with increased NT (9,15-16). In a large study by Kagan et al. (17), evaluating 11,315 patients with increased NT, the chromosomal anomaly was found in 19% of the patients. In a study by Boutot et al. (18), evaluating 398 patients, invasive diagnostic testing was performed in 87% of the patients, and chromosomal

anomalies were detected in 37.4% of the patients, the most common being Trisomy 21. In another study in which a similar chromosomal anomaly rate was detected as our study, invasive diagnostic testing was performed on 50% of the patients, and a 12.1% rate of chromosomal anomaly was detected, the most common being Trisomy 21 (19). We thought that the majority of patients did not undergo invasive diagnostic testing due to the socio-cultural structure and religious beliefs of our region. In our study, karyotype analysis could not be performed on 16.6% (n=14) of the patients. We thought that the 14 patients for whom karyotype analysis could not be performed affected the chromosomal anomaly detection rate and that if karyotype analysis could be performed on these patients, we could detect a higher chromosomal anomaly rate. We could not identify a specific syndrome in our study. However, due to multiple anomalies, fetal loss was encountered. We thought that it might be among the patients who died. Additionally, only conventional karyotyping is performed in our hospital. Chromosomal microarray analysis (CMA) and other advanced genetic tests were not studied. In a meta-analysis in which fetuses with increased NT and normal karyotypes were evaluated, the chromosomal anomaly detection rate of CMA was found to be 5% (20). If CMA could have been studied in our study, more chromosomal anomalies could have been detected.

Increased NT is also associated with poor pregnancy outcomes such as fetal structural anomaly and loss (6-11). In our study, prenatal and postnatal results were evaluated together and fetal structural anomaly, most commonly cardiac anomaly, was detected in 29.4% (n=25) of the patients. Structural anomalies, most commonly cardiac and then facial anomalies, were detected in 21% (n=13) of patients with normal karyotypes. In a study in China where 264 patients with increased NT were evaluated, fetal anomalies were detected in 22.3% of the patients, the most common being hydrops, followed by cardiac anomalies (19). Senat et al. (15) detected structural anomalies in 27% of patients with increased NT. Boutot et al. (18) detected structural anomalies in 28.7% of the patients, the most common being cardiac and then urogenital anomalies. In another study, structural anomaly was detected in 9% of 834 cases with increased NT and normal karyotype (11). In our study, it was observed that the 21% (n=13) fetal structural anomaly rate detected in patients with normal karyotypes increased to 29.4% (n=25) with the addition of patients with abnormal karyotypes or unknown karyotypes. It was thought that this was due to fetal structural anomalies that often accompany chromosomal anomalies. Our fetal anomaly detection rates were found to be compatible with the literature.

In our study, 74.1% (n=63) of the patients' pregnancies ended in live births and 25.9% (n=22) in fetal loss. It was found that pregnancy loss was 23.1% (n=3) in patients with normal karyotype if accompanied by fetal structural anomaly, and 2% (n=1) if not accompanied by fetal structural anomaly. In a study, fetal loss rates of up to 13% were reported in patients with increased NT, especially if they were accompanied by structural anomalies (21). In another study evaluating pregnant women with normal karyotype and increased NT, fetal loss was found to be 7.14% (22). In another study evaluating pregnant women with normal karyotype and increased NT, total fetal loss was found to be 15.8%. In the same study, the fetal loss rate was found to be 58.3% in the group with fetal structural anomaly and 3.5% in the group without fetal structural anomaly (23). In our study, consistent with the literature, it was observed that poor pregnancy outcomes became evident when the presence of structural anomalies was added to the increase in NT.

In our study, NT and NT MoM values were found to be significantly higher in patients with fetal anomaly than in patients without fetal anomaly, and in patients with abnormal karyotype compared to patients with normal karyotype. NT and NT MoM values were found to be significantly higher in patients with fetal loss or abnormal newborn examination compared to the patients without. Similar to our study, Uysal et al. (23) found NT and NT MoM values to be significantly higher in the group with an increase in NT and a fetal anomaly or pregnancy loss compared to the group without a fetal anomaly or pregnancy loss (23). In another study evaluating the pregnancy outcomes of increased NT, it was found that 73% of fetuses with normal karyotypes were in the group with the lowest NT value (11). In a study in which 720 patients with increased NT and normal karyotype were evaluated, significant results were found indicating that as the NT value increased, the risk of fetal anomaly, cardiac anomaly, hydrops fetalis, abortion, and intrauterine fetal death increased (24). Our findings support that, similar to other studies in the literature, poor pregnancy outcomes increase as NT and NT MoM values increase.

CONCLUSION

NT evaluation is very important in antenatal follow-up. In our study, as in other studies in the literature, NT increase is associated with chromosomal anomalies, fetal structural anomalies, and poor pregnancy outcomes. We observed that as NT or NT MoM values increased, the risk of chromosomal anomaly, structural anomaly, and poor pregnancy outcomes in the fetus increased. It was found that detecting fetal anomalies in patients with normal karyotypes increased poor pregnancy outcomes. Increased NT increases the risk of fetal anomalies. For these reasons, fetal anomaly screening and fetal ECHO should be recommended in patients with increased NT, even if a normal karyotype is detected.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Van Yüzüncü Yıl University (18.11.2022, 11-27).

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Medical Informatics as a Concept and Field-Based Medical Informatics Research: The Case of Turkey

Kavramsal Olarak Tıbbi Bilişim ve Alan Bazlı Tıp Bilişimi Araştırmaları: Türkiye Örneği

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ABSTRACT

Aim: This study aimed to evaluate the position of Turkey in the field of Medical Informatics and assess the general structure of research by analyzing Medical Informatics research with bibliometric methods.
Material and Methods: In this study, we conducted a bibliometric analysis of research and review articles generated between 1980 and 2023 from the Web of Science bibliometric data source, utilizing bibliometric methods through the R bibliometrix tool and VosViewer.
Results: In the field of medical informatics research in Turkey, the country holds the 27th position with 905 articles, 15,610 citations, and an impressive impact factor of 51, along with an average citation rate of 17.25 per article, based on bibliometric analysis conducted between 1980 and 2023. Notable institutions in this field include Middle East Technical University, Hacettepe University, and Selçuk University. The prominent research topics encompass "neural network(s), machine learning, support vector, health care, decision support, deep learning, EEG signals, classification accuracy," reflecting the areas of intensive investigation.
Conclusion: In Turkey, the field of medical informatics has lagged slightly behind basic engineering sciences or medical sciences. The domain exhibits a multidisciplinary structure intersecting with various engineering fields such as computer science, software engineering, industrial engineering, artificial intelligence engineering, and electronic engineering. To enhance productivity in this field, greater collaboration with other research areas can be pursued. Additionally, it is recommended to urgently establish four-year undergraduate programs specifically dedicated to medical informatics or health informatics at universities.
Keywords: Turkey; medical informatics; scientific productivity; bibliometrics; citation analysis.

ÖZ

Amaç: Bu çalışmada, Tıp Bilişimi araştırmalarını bibliyometrik yöntemler ile analiz ederek Türkiye'nin Tıp Bilişimi alanındaki konumunu ve araştırma genel yapısını değerlendirmeyi amaçlanmıştır.
Gereç ve Yöntemler: Bu çalışmada, R bibliyometrix ve VosViewer aracılığı ile Web of Science bibliyometrik veri kaynağından 1980 ile 2023 yılları arasında üretilen araştırma ve derleme türündeki makaleler bibliyometrik yöntemler ile analiz edilmiştir.
Bulgular: Türkiye tıp bilişimi araştırma alanında, 1980 ile 2023 yılları arasında yapılan bibliyometrik analize göre, 905 makale, 15610 atıf ve ilgili makalelere verilen 17,25 atıf ortalaması, 51 gibi yüksek bir etki değeri ile 27. sırada yer almaktadır. İlgili alanda öne çıkan kurumlar arasında, Ortadoğu Teknik Üniversitesi, Hacettepe Üniversitesi ve Selçuk Üniversitesi bulunmaktadır. Öne çıkan araştırma konuları arasında yoğun araştırma alanlarını yansıtan "sinir ağları, makine öğrenimi, destek vektörü, sağlık hizmetleri, karar desteği, derin öğrenme, EEG sinyalleri, sınıflandırma doğruluğu" yer almaktadır.
Sonuç: Türkiye'de tıp bilişimi uzmanlık alanı temel mühendislik bilimlerine veya tıp bilimlerine göre biraz daha geride kalmıştır. Alanın bilgisayar bilimleri, yazılım mühendisliği, endüstri mühendisliği, yapay zekâ mühendisliği ve elektronik mühendisliği gibi pek çok farklı mühendislik alanı ile kesişen multidisipliner bir dokusu mevcuttur. Bu alanda daha etkin üretkenlik için alanın diğer araştırma alanları ile daha fazla ilişkiye geçilebilir. Ayrıca tıp bilişimi veya sağlık bilişimine ilişkin ivedi olarak dört yıllık lisans programlarının üniversitelerde kurulması önerilmektedir.
Anahtar kelimeler: Türkiye; tıp bilişimi; bilimsel üretkenlik; bibliyometri; atıf analizi.

INTRODUCTION

The adoption rate of novel information and informatics technologies, such as artificial intelligence, machine learning, big data, blockchain, cloud computing, wearable and implantable technologies, virtual and augmented reality technologies, and mobile health applications is on the rise in the healthcare sector. These technologies offer numerous new advantages and opportunities, advancing the healthcare sector. However, the need for complex hardware and training of the medical staff to use these technologies is also increasing over time (1). The discipline of medical informatics plays a significant role in this context.

According to Masic (2), after its appearance around the 1950s, medical informatics is recognized as a scientific discipline that deals with the theory and practice of information processes in medicine, incorporating information technologies and data communication, particularly focusing on computers as a significant tool for information processing and analysis. Medical informatics encompasses the examination and implementation of methods aimed at improving the management (i.e., security, storage, cleaning) and analysis of patient data, clinical information, population data, and other information related to patient care as well as developing medical decision support systems (3). This represents a field that aims to enhance patient care through the management of information in the healthcare sector and the utilization of technological advancements.

Lincoln (4) highlights the reliance of medical informatics on a range of general disciplines, including logic, mathematics, computer science, and behavioral sciences as well as focused fields like decision theory, artificial intelligence, systems analysis, and industrial psychology. Haux (5) also underscores the interdisciplinary nature of medical informatics and asserts that medical informatics is instrumental in the future of medicine and healthcare services. The progression of medical informatics hinges on the presence of well-educated healthcare professionals specializing in medical informatics. These professionals may encompass doctors, nurses, healthcare managers, medical informatics specialists, or individuals from related fields.

In the delivery of high-quality and efficient healthcare services, medical informatics bears a significant responsibility for advancing human health through innovative research in health and computer sciences related to biomedicine (6). Therefore, medical informatics education is crucial for medical and healthcare services students, who are the future of the healthcare profession. In addition, medical informatics provides valuable learning resources for the continuous professional development of clinicians to keep up with the rapid advancements in the field (7). Medical informatics education is also essential for other healthcare personnel at all levels of care delivery. Therefore, in Europe, many universities have curricula in the field of medical informatics. For instance, in Germany, there are medical informatics undergraduate programs at 14 universities (8). In Turkey, there is currently no undergraduate program in the field of medical informatics. However, many universities in the country are opening graduate-level programs and establishing departments in the related field. This highlights a growing attendance and interest in education on medical informatics.

There is a natural feedback loop between advances in medical informatics and its education: the former provides education materials for the latter and the latter contributes to the training of the professionals who achieve the former. Therefore, understanding the standing of a country in advancing medical informatics has direct implications for the country's strengths and needs in medical informatics education. Thus, our study's primary objective is to examine the volume of medical informatics research conducted by researchers in Turkish institutions using bibliometric methods. Our bibliometric analysis provides a detailed overview of the bibliographic structure of publications, the positioning of Turkish researchers in the global landscape, and the prominent topics addressed in the publications, which will shed light on the need for improvements in medical informatics research and education in Turkey.

The examination of relevant literature reveals several review studies focusing on the development of health informatics in Turkey. These studies cover various aspects of health informatics; the academic evaluation of the development of health informatics in Turkey, discussions on health informatics and the digitization of hospitals in Turkey, evaluation of the development of the health informatics infrastructure in the public domain in Turkey, discussions on nursing informatics within the field of health informatics, assessments of legal issues in health informatics, evaluations of e-health applications, discussions on the importance of health informatics systems in increasing hospital efficiency, and highlighting the significance of information systems for the healthcare sector (1,9-14). In addition, there are several international bibliometric articles in the field of medical informatics, which extensively study certain relevant topics including mobile health, artificial intelligence in healthcare, augmented reality in medicine, natural language processing in medical research, highly cited articles in the field of healthcare sciences and services, telemedicine, machine learning, virtual reality, and augmented reality (15-25).

Nevertheless, the above literature reviews and international bibliometric studies do not address issues related to the scientific productivity of Turkish institutions in the medical informatics field. Our study aims to fill this gap by conducting a bibliometric analysis of national and international publications of the Web of Science (WoS) sources. Our results provide insights into the general structure of scientific productivity among Turkish researchers in the field of medical informatics. Our study may be of value to the existing and new researchers in the field for highlighting prominent medical informatics researchers, institutions, countries, journals, and works in the field.

MATERIAL AND METHODS

In this study, bibliometric data were retrieved from the WoS (Clarivate Analytics Corporations, London, UK) bibliometric data source on November 4, 2023, using the query specified for the field of medical informatics (wc="Medical Informatics" and TURKIYE or TURKEY, Countries/Regions). The number of articles produced in the field of medical informatics is 95,948 in all years. When examining the scientific productivity of studies with

Turkish affiliations by years, articles addressed to two different country names, Turkiye (*f*:79) and Turkey (*f*:826), were filtered. A total of 905 articles, categorized as research articles and reviews, published in English between 1980 and 2023, were analyzed using bibliometric methods. The reason why only article and review article types are included in the analysis is that the relevant document types have richer data features for bibliometric analysis than book chapters, proceeding papers, and letters. Within the scope of the analyses, various statistics were obtained from the dataset, including the intensity of publications over the years, country distributions, most preferred and cited journals, leading authors and institutions in the field, and keyword distributions.

Journals in the WoS belong to the Arts & Humanities Citation Index (A&HCI), Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), or Emerging Sources Citation Index (ESCI). The journals in these indexes are widely recognized by the global scientific community and are considered a priority in academic performance criteria. Therefore, it is reasonable to assume that a significant academic advance in the medical informatics field would eventually lead to a publication in a journal in these indexes. Thus, focusing on the WoS bibliometric database for our study enables access to a high-quality and rich dataset for capturing prominent research studies shaping this field.

VOSviewer and Biblioshiny (within the R Bibliometrix) programs were utilized during the analysis process, particularly for constructing network structures and detailed tables based on the demographic characteristics of articles. VOSviewer, Bibexcel, Citespace, HistCite, Pajek, UCINET, VIVO, and Sci2 are software applications commonly employed for social network analyses (26). These software tools are for creating and visualizing bibliometric networks encompassing journals, researchers, or individual publications, which can be constructed based on citation, bibliographic coupling, co-citation, or co-authorship relationships (27). Biblioshiny, on the other hand, is another software designed with the R programming language, operating with the bibliometrix library (28). Additionally, a web-based application was developed using Hypertext Preprocessor (PHP) programming language to parse WoS data and transfer it to a database designed in Oracle. This facilitated the filtering of studies based on summary information, title, keywords, and the titles of the studies. Consequently, text analyses were also conducted as part of the study.

RESULTS

Medical Informatics Research Area General View and Turkey

Throughout all years, international institutions produced 145,523 scientific contributions of various forms in medical informatics: Original articles (*f*:90,527, 62.20%), proceeding papers (*f*:41,254, 28.34%), editorial materials (*f*:5,871, 4.03%), review articles (*f*:5,421, 3.72%), book chapters (*f*:3,858, 2.65%), meeting abstracts (*f*:2,816, 1.93%), letters (*f*:1,671, 1.14%) and others (*f*:3,559, 2.44%). Our study considers only original articles and review articles, totaling 95,948 contributions. The productivity of relevant articles by country is shown in Table 1.

When examining the country-specific scientific productivity, we combined research affiliated with the country names of "Turkiye" (*f*:79) and "Turkey" (*f*:826) to reflect the recent name change. Among all countries with scientific contributions to medical informatics, Turkey ranks 27th (Table 1) with 905 articles, which were all published in English.

Table 2 shows the distribution of the 1271 contributions affiliated with Turkey according to publication types. Original articles and proceeding papers together constitute a vast majority of all contributions (i.e., 96.30%). The review articles' contributions follow the top three with a much lower proportion of 1.57%, while the proportion of the remaining contribution types is limited to 4.24%. It should be noted that WoS can classify the same document into more than one type. For example, an article can also be classified as a proceeding paper or book chapter.

As mentioned before, original article and review article types were examined in our study. Figure 1 illustrates a citation analysis showing that a total of 905 articles affiliated with Turkey have received 15,610 citations, and these documents have a high impact value of 51. The document average age is 7.44. Articles in the field of medical informatics affiliated with Turkey have been referenced by

Table 1. Country settings in original articles and review articles on medical informatics

Rank	Country	n	% of 95,948
1	USA	35,718	37.61
2	ENGLAND	9,370	9.86
3	CHINA	7,370	7.76
4	GERMANY	7,370	7.76
5	CANADA	6,113	6.43
6	AUSTRALIA	5,062	5.33
7	NETHERLANDS	4,428	4.66
8	SPAIN	3,163	3.33
9	ITALY	3,160	3.32
10	FRANCE	3,062	3.22
11	SOUTH KOREA	2,384	2.51
12	INDIA	2,223	2.34
13	JAPAN	2,212	2.33
14	SWEDEN	2,123	2.23
15	SWITZERLAND	1,994	2.10
16	TAIWAN	1,922	2.00
17	BELGIUM	1,503	1.56
18	AUSTRIA	1,303	1.35
19	SCOTLAND	1,211	1.26
20	DENMARK	1,202	1.25
21	BRAZIL	1,142	1.19
22	NORWAY	1,128	1.17
23	IRAN	1,116	1.16
24	SINGAPORE	1,116	1.16
25	FINLAND	1,113	1.16
26	GREECE	1,088	1.13
27	TURKEY	905	0.94
28	ISRAEL	852	0.88
29	PORTUGAL	838	0.87
30	NEW ZEALAND	642	0.66

Table 2. The distribution of documents by type

Rank	Document Type	n	% of 1,271
1	Original Article	885	69.63
2	Proceeding Paper	347	27.30
3	Review Article	20	1.57
4	Early Access	15	1.18
5	Book Chapters	12	0.94
6	Correction	10	0.78
7	Editorial Material	10	0.78
8	Letter	5	0.39
9	Meeting Abstract	1	0.07
10	Note	1	0.07

30,028 sources. There is an increasing productivity in article publications over the years. When the documents associated with the field of medical informatics from Turkey are analyzed according to all document types, 2018 was the most productive year with 140 documents, showing a 212% increase compared to the previous year (66 documents were produced in 2017). When focusing on 2018, the primary reason for this increase can be attributed to documents in the "proceedings paper" category. The significant impact of the Medical Technologies National Congress (TIPTEKNO), held in 2018 and indexed by the Web of Science Conference Proceedings Citation Index - Science (CPCI-S), is noted.

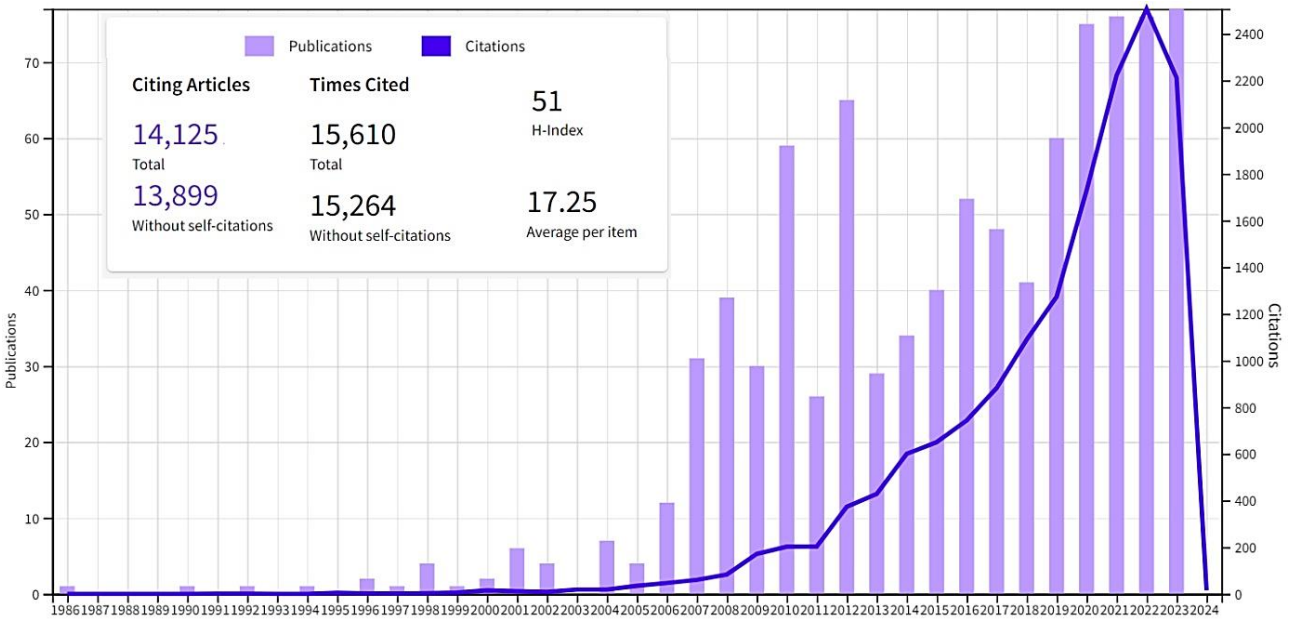

Figure 1. Publications cited over time

The distribution of the 905 scientific contributions in terms of the journal indexes is as follows: SCI-Expanded (*f*:849), SSCI (*f*:155), ESCI (*f*:38). Additionally, 21.76% of the studies have been published as open access. The years 2023 (*f*:77), 2022 (*f*:76), 2021 (*f*:76), and 2020 (*f*:75) stand out as the most productive years. In terms of funding sources, the top ten institutions that have supported the publications affiliated with Turkey are, in order: Turkey Scientific and Technological Research Institution (TUBITAK, *f*:80), Selcuk University (*f*:20), United States National Institutes of Health (NIH, *f*:20), United States Department of Health Human Services (*f*:20), European Union (EU, *f*:17), Bogazici University (*f*:11), Istanbul University (*f*:11), Akdeniz University (*f*:10), EU Joint Research Centre (*f*:10), Yildiz Technical University (*f*:6).

Authors, Institutions, and Country Analyses

Considered articles affiliated with Turkey have been produced by researchers from 72 different countries, 997 different institutions, and 2666 researchers. The top five most productive researchers, their institutional information, h-index (HI), Average Citation Per Document (ACPD), total citations (TC), and article count (N) are shown: Sadik Kara (Erciyes University, *f*:9, ACPD:12.00, TC:228, N:19), Sengur Abdulkadir (Fırat University, *f*:14, ACPD:35.72, TC:643, N:18), Inan Güler (Gazi University, *f*:7, ACPD:36.77, TC:478, N:13), Asuman Doğaç (Middle East Technical University, *f*:9, ACPD:19.27, TC:212, N:11), Fatma Latifoğlu (Erciyes University, *f*:6, ACPD:12.82, TC:141, N:11). The number of single-authored documents is 71, the international co-authorship rate is 25.76%, and the co-authors per document value is 3.88.

Figure 2, shows the collaboration network (i.e., collaborating researchers, institutions, and countries in medical informatics publications affiliated with Turkey. The top ten countries with the highest rate of collaboration with Turkish institutions for publishing articles in medical informatics are the USA (*f*:97, 10.71%), England (*f*:24, 2.65%), Germany (f:22, 2.43%), Canada (*f*:21, 2.32%), France (*f*:20, 2.21%), Netherlands (*f*:20, 2.21%), Australia (*f*:17, 1.87%), Italy (*f*:17, 1.87%), Spain (*f*:15, 1.65%), and India (*f*:14, 1.54%). The most intensively collaborated institutions in the United States are the University of Wisconsin (*f*:9), Harvard University (*f*:8), and Stanford University (*f*:6), which are the pioneering institutions in this field. In Turkey, the institutions that have collaborated intensively with the aforementioned universities are Akdeniz University (*f*:6) and Hacettepe University (*f*:12).

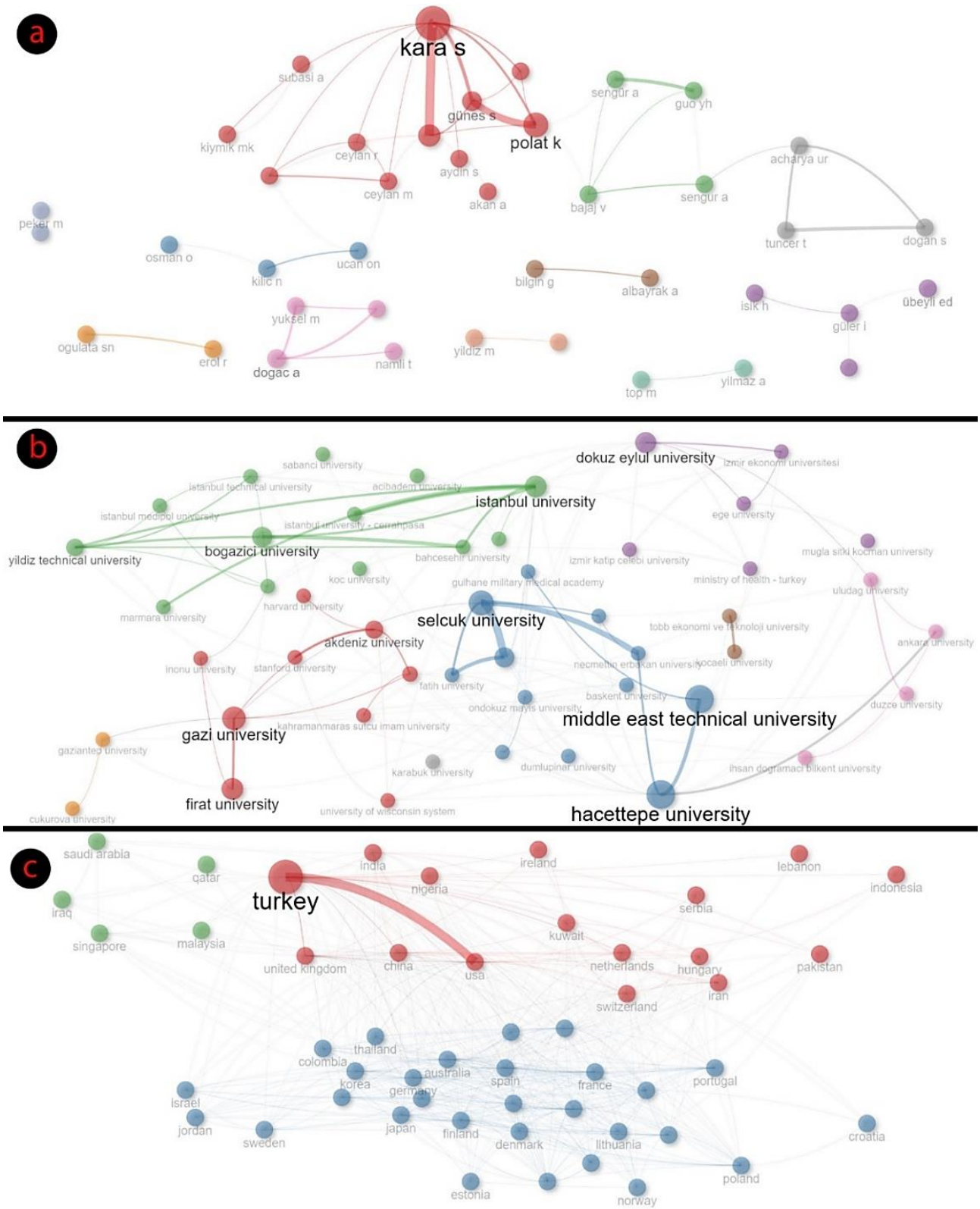

Figure 2. Author (a), affiliation (b), and country (c) collaboration analyses

The top five institutions with the most intensive international cooperation among all institutions are Fırat University (*f*:23), Middle East Technical University (*f*:22), Hacettepe University (*f*:21), Akdeniz University (*f*:12), Bogazici University (*f*:11), respectively. The institutions with the most intense collaboration in the United Kingdom are the University of London (*f*:8), Queen Mary University London (*f*:3), and University of Southampton (*f*:3). In Germany, the most intensively collaborated institutions are Institut National De La Sante Et De La Recherche Medicale (*f*:3) and Hannover Medical School (*f*:3).

Table 3 shows the Turkish institutions that produce the most article publications in medical informatics. The top five institutions are, respectively, Middle East Technical

Institution (*f*:65), Hacettepe University (*f*:63), Selcuk University (*f*:47), Fırat University (*f*:46), and Gazi University (*f*:46). The vast majority of the below institutions have their faculties of medicine as the presence of a medical faculty significantly improves the productivity of studies in medical informatics. Interestingly, the Middle East Technical University and Bogazici University, ranked first and seventh, respectively, do not have medical faculties. This is an important issue to be examined. When the scientific productivity of the Middle East Technical University is examined in detail, the influence of programs such as the faculty of engineering (*f*:27), department of computer engineering (*f*:18), graduate school of informatics (*f*:6), faculty of arts and sciences (*f*:5), department of electrical and electronics engineering (*f*:5), and department of statistics (*f*:5) becomes apparent.

Journal Analysis

Table 4 illustrates the journals publishing most medical informatics articles from Turkey. The 905 medical informatics articles are published in 57 different journals. Most of the top 20 journals have high HI and citations per document. These journals are in the first and second quartiles journals of medical informatics research area in WoS categories. These journals are mostly indexed in the Science Citation Index Expanded (SCIE), i.e., 19 out of the

Table 3. The distribution of articles and reviews addressed to Turkey by institutions

Rank	Affiliation	HI	ACPD	TC	N	% of 905
1	Middle East Technical University	15	9.95	647	65	7.18
2	Hacettepe University	17	23.24	1,464	63	6.96
3	Selcuk University	17	15.21	715	47	5.19
4	Fırat University	24	32.59	1,499	46	5.08
5	Gazi University	16	24.85	1,143	46	5.08
6	Dokuz Eylül University	15	19.02	780	41	4.53
7	Bogazici University	13	24.37	926	38	4.19
8	Erciyes University	13	13.56	488	36	3.97
9	Akdeniz University	9	6.79	224	33	3.64
10	Istanbul University	12	23.79	785	33	3.64
11	Yıldız Technical University	11	11.93	322	27	2.98
12	Sakarya University	9	13.27	345	26	2.87
13	Ege University	12	21.24	531	25	2.76
14	Tobb Ekonomi ve Teknoloji University	11	24.96	574	23	2.54
15	Cukurova University	12	19.50	429	22	2.43
16	Baskent University	9	9.48	199	21	2.32
17	Istanbul Technical University	10	20.71	435	21	2.32
18	Bahcesehir University	10	35.70	714	20	2.21
19	Karadeniz Technical University	9	14.11	254	18	1.98
20	Istanbul University Cerrahpasa	9	31.88	542	17	1.87

HI: h-index, ACPD: average citation per document, TC: total citations, N: article count

Table 4. Top 20 journals publishing most medical informatics articles affiliated with Turkey

Rank	Publication Title	5JIF	Index	Q	HI	ACPD	TC	N	% 905
1	Journal of Medical Systems	5.200	SCIE	Q2	33	17.86	3,840	215	23.75
2	Computer Methods and Programs in Biomedicine	6.100	SCIE	Q2	33	49.39	7,952	161	17.79
3	Medical Biological Engineering Computing	3.100	SCIE	Q3	17	10.39	1,018	98	10.82
4	Biomedical Engineering Biomedizinische Technik	1.600	SCIE	Q4	6	3.39	129	38	4.19
5	Computers Informatics Nursing	2.000	SCIE	Q4	11	8.81	317	36	3.97
6	Artificial Intelligence in Medicine	7.400	SCIE	Q1	13	21.10	633	30	3.31
7	IEEE Journal of Biomedical and Health Informatics	7.700	SCIE	Q1	13	34.24	993	29	3.20
8	Journal of Evaluation in Clinical Practice	2.500	SCIE	Q4	9	8.54	239	28	3.09
9	Journal of Biomedical Informatics	6.900	SCIE	Q2	14	28.54	742	26	2.87
10	IEEE Transactions on Information Technology in Biomedicine	2.873	SCIE	Q1	12	37.58	714	19	2.09
11	International Journal of Medical Informatics	5.400	SCIE	Q2	8	9.70	194	20	2.21
12	BMC Medical Informatics and Decision Making	3.900	SCIE	Q3	8	11.31	181	16	1.76
13	Health and Technology	2.300	ESCI	Q4	6	4.79	67	14	1.54
14	Health Information Science and Systems	5.700	SCIE	Q1	9	33.46	435	13	1.43
15	Journal of Medical Internet Research	7.600	SCIE	Q1	9	18.15	236	13	1.43
16	International Journal of Technology Assessment in Health Care	2.600	SCIE	Q3	6	6.75	81	12	1.32
17	Methods of Information in Medicine	2.500	SCIE	Q4	8	12.58	151	12	1.32
18	Digital Health	4.400	SCIE	Q2	2	2.00	24	12	1.32
19	Informatics for Health Social Care	2.900	SCIE	Q4	5	10.80	108	10	1.10
20	Health Informatics Journal	3.000	SCIE	Q3	4	5.10	51	10	1.10

JIF: journal impact factor, SCIE: science citation index expanded, ESCI: emerging sources citation index, Q: quartile, HI: h-index, ACPD: average citation per document, TC: total citations, N: article count

top 20 journals are indexed in SCIE. It is seen that five journals are in the first quartile, five journals are in the second quartile, four journals are in the third quartile, and finally, six journals are in the fourth quartile. The top three journals are the Journal of Medical Systems (*f*:215, 23.75%), Computer Methods and Programs in Biomedicine (*f*:161, 17.79%), and Medical Biological Engineering Computing (*f*:98, 10.82%). These three journals have published around one out of every two medical informatics articles from Turkey (474 documents, 52.37% of total publications).

Table 5 displays the top fifteen articles with the highest citations in the field of medical informatics with Turkey affiliation. Eight of the top fifteen articles with the highest citations were published in Computer Methods and Programs in Biomedicine, while each of the remaining seven was published in a different journal. Also, note that six of the top fifteen articles were on developing general-purpose bioinformatics methodology, while the remaining nine were on informatics analysis and methods for specific medical conditions.

Research Areas and Content Analysis

Medical informatics articles affiliated with Turkey have been associated with 21 different research areas listed in the Web of Science. The top five of these research areas are computer science interdisciplinary applications (*f*:379,

Table 5. The first 15 articles with the highest number of citations published in the field of medical informatics research in Turkey

Rank	Authors / Title / Journal	5JIF	Q	Years	TC
1	Stijnen T, Hamza TH, Ozdemir P Random effects meta-analysis of event outcome in the framework of the generalized linear mixed model with applications in sparse data Statistics in Medicine	2.700	Q4	2010	418
2	Sakar BE, Isenkul ME, Sakar CO, Sertbas A, Gurgen F, Delil S, Apaydin H, Kursun O Collection and analysis of a Parkinson speech dataset with multiple types of sound recordings IEEE Journal of Biomedical and Health Informatics	7.700	Q1	2013	329
3	Subasi A, Ercelebi E Classification of EEG signals using neural network and logistic regression Computer Methods and Programs in Biomedicine	6.100	Q1	2005	322
4	Guler I, Ubeyli ED Multiclass support vector machines for EEG-signals classification IEEE Transactions on Information Technology in Biomedicine	2.873	Q1	2007	245
5	Yildirim O, Baloglu UB, Tan RS, Ciaccio EJ, Acharya UR A new approach for arrhythmia classification using deep-coded features and LSTM networks Computer Methods and Programs in Biomedicine	6.100	Q1	2019	188
6	Sen B, Peker M, Çavusoglu A, Çelebi FV A comparative study on classification of sleep stage based on EEG signals using feature selection and classification algorithms Journal of Medical Systems	5.200	Q2	2014	181
7	Can YS, Arnrich B, Ersoy C Stress detection in daily life scenarios using smartphones and wearable sensors: A survey Journal of Biomedical Informatics	6.900	Q2	2019	174
8	Ozcift A, Gulten A Classifier ensemble construction with rotation forest to improve medical diagnosis performance of machine learning algorithms Computer Methods and Programs in Biomedicine	6.100	Q1	2011	161
9	Turkyilmazoglu M Single phase nanofluids in fluid mechanics and their hydrodynamic linear stability analysis Computer Methods and Programs in Biomedicine	6.100	Q1	2020	160
10	Kutlu Y, Kuntalp D Feature extraction for ECG heartbeats using higher order statistics of WPD coefficients Computer Methods and Programs in Biomedicine	6.100	Q1	2012	152
11	Dokur Z, Olmez T ECG beat classification by a novel hybrid neural network Computer Methods and Programs in Biomedicine	6.100	Q1	2001	152
12	Cinsdikici MG, Aydin D Detection of blood vessels in ophthalmoscope images using MF/ant (matched filter/ant colony) algorithm Computer Methods and Programs in Biomedicine	6.100	Q1	2009	143
13	Guvenir HA, Demiroz G, Ilter N Learning differential diagnosis of erythemato-squamous diseases using voting feature intervals Artificial Intelligence in Medicine	7.400	Q1	1998	139
14	Deniz E, Sengur A, Kadiroglu Z, Guo Y, Bajaj V, Budak U Transfer learning-based histopathologic image classification for breast cancer detection Health Information Science and Systems	5.700	Q2	2018	133
15	Hariharan M, Polat K, Sindhu R A new hybrid intelligent system for accurate detection of Parkinson's disease Computer Methods and Programs in Biomedicine	6.100	Q1	2014	132

41.87%), health care sciences services (*f*:379, 41.54%), and engineering biomedical (*f*:341, 37.68%). As observed, research has been conducted in various domains ranging from technical fields such as materials science, computer science, information science, and artificial intelligence to applied and practical fields including nursing, health policy services, library science, and psychiatry (Table 6).

The most prominent analysis methods and techniques in the research associated with the research area of computer science interdisciplinary applications are listed as follows: Machine learning, deep learning, neural network, learning algorithms, decision support, feature selection, automatic segmentation, computer simulation, cell segmentation, classification algorithms, random forest (30-47).

In these studies, blood pressure, Doppler signals, artery Doppler, imaging (medical, histopathological, ECG, MRI), care results, human resources management (HRM) data, and surface electromyography (sEMG) signals have been the data or data types analyzed in research and examined for health studies. In computer science interdisciplinary applications research area, considered medical conditions include Parkinson's disease, brain tumor, breast cancer, Alzheimer's disease, cancer diagnosis, colorectal, and glaucoma disease (31,32,35,37,41,48-55). Cancer has emerged as an intensively studied disease in the field of medical informatics.

The research field of engineering biomedical has emerged as another closely related domain to the field of medical informatics. In the engineering biomedical research area, methods and techniques such as neural networks, clustering, segmentation, feature extraction, classification, learning algorithms, machine learning, and deep learning have been extensively utilized, similar to their intensive use in the Computer Science Interdisciplinary Applications field.

In the field of Health Care Sciences Services, the following topics have been prominently featured and extensively studied: EEG signals classification, detection of diseases by processing clinical data, medical decision support systems, classification methods for different purposes, attitudes of health professionals, some software tools and programmable tools, social media use for health topics (15,56-61). Intensively conducted studies in this research area have predominantly focused on disease detection and prediction.

Although it is not among the top three, it is the focal point of medical informatics articles related to the field of nursing, where there is an intense scientific productivity in the relevant field, the focus is not limited to the development of information systems or software but also on measuring the impact or experience of the developed applications on users. Some prominent topics include the effect of problematic internet use, social appearance anxiety, and social media use, web-based education for students, nurses' attitudes toward computers and computer use, nursing students' mobile technology use, and decision support systems (62-67).

DISCUSSION

A noteworthy aspect of bilateral collaboration is the diaspora effect in international collaboration, i.e., the Turkish diaspora in the scientific world plays a crucial role in the internationalization of Turkish researchers. When the names of researchers are carefully analyzed, it is observed that our citizens who worked at prestigious international institutions have successfully conducted rigorous research in these countries, and then they have continued their collaborations with researchers in these prestigious institutions upon returning to Turkey. This is a very important and valuable finding highlighting the importance of doing graduate studies abroad or increasing support for sending Turkish researchers abroad for postdoctoral research to achieve better results in research

Table 6. The other areas related to medical informatics research fields located in Turkey

Rank	Web of Science Categories	n	% of 905
	Medical Informatics	905	100
1	Computer Science Interdisciplinary Applications	379	41.87
2	Health Care Sciences Services	376	41.54
3	Engineering Biomedical	341	37.68
4	Computer Science Theory Methods	162	17.90
5	Mathematical Computational Biology	160	17.68
6	Computer Science Information Systems	93	10.27
7	Public Environmental Occupational Health	39	4.30
8	Nursing	36	3.97
9	Computer Science Artificial Intelligence	32	3.53
10	Medicine General Internal	30	3.31
11	Health Policy Services	22	2.43
12	Statistics Probability	13	1.43
13	Medicine Research Experimental	8	0.88
14	Information Science Library Science	7	0.77
15	Pharmacology Pharmacy	3	0.33
16	Computer Science Cybernetics	1	0.11
17	Computer Science Software Engineering	1	0.11
18	Materials Science Biomaterials	1	0.11
19	Medical Laboratory Technology	1	0.11
20	Psychiatry	1	0.11
21	Psychology Clinical	1	0.11

outcomes and improve Turkey's scientific standing in medical informatics. If our researchers abroad want to stay, their stay should be supported given a good plan for further network and capacity building, and positive support can be provided for them to maintain their ties with Turkey.

It is also important to highlight the frequent collaboration between Middle East Technical University and Hacettepe University, which has an internationally well-known faculty of medicine. This may imply that highly technical institutions may rely on their strong methodological backgrounds to develop or utilize advanced methods of analyzing medical data from their collaborating institutions to conduct high-quality medical informatics research. This could be an ideal approach for promoting medical informatics research in Turkish institutions without medical schools, for which proper incentives can be introduced by funding agencies and scientific regulation offices. In addition, this study shows that 68.61% of the health studies addressed in Turkey were not supported. It can be said that this value is quite low for a research field such as medical informatics, which has direct links with two critical and current fields such as health and information sciences.

Bradford's Law of Distribution defines the distribution of articles or publications on a specific topic across journals. Garfield characterized Bradford's Law as follows: "If you want to compile a bibliography on a specific subject, you will find that a small core group of journals contains about one-third of the articles published in that subject or discipline, which we always refer to as the significant core." (29). From this perspective, the core journals for researchers in the field of medical informatics in Turkey can be identified as the Journal of Medical Systems and Computer Methods and Programs in Biomedicine.

The homogeneous relationship of the articles in the field of medical informatics produced in Turkey with computer science, statistics, health sciences, and even materials science and psychiatry research fields can be considered as strong evidence of the multidisciplinary nature of the field. This can be illustrated by an analysis of keywords in the considered studies. The distribution of keywords used by researchers in their studies includes machine learning (*f*:62), deep learning (*f*:48), classification (*f*:38), COVID-19 (*f*:23), feature extraction (*f*:23), electroencephalography (EEG, *f*:22), Turkey (*f*:19), feature selection (*f*:17), artificial intelligence (*f*:15), data mining (*f*:15), artificial neural networks (*f*:13), convolutional neural networks (*f*:12), image processing (*f*:12), neural networks (*f*:12), transfer learning (*f*:12), support vector machines (*f*:11), fuzzy logic (*f*:10), and image segmentation (*f*:10). It is observed that the keywords used in the considered articles have a significant influence from computer and decision sciences. The need for the systematic processing of data, information, and knowledge in medicine and health services continues. Moreover, due to its cross-sectional nature covering most disciplines in medicine and health sciences, medical Informatics should be considered a critical field for the future of medicine and health services.

When the abstracts of the studies are examined according to the trend topic analysis, the situation depicted in Figure 3 emerges. It is evident here that technological transformation is directly reflected in the literature. While smart card technology was intensively used in the 2000s, in recent years, virtual reality (21,68) stands out in health informatics studies affiliated with Turkey. As seen in Figure 3a and Figure 3b, the topic headings of neural networks, decision support, decision science, machine learning, and deep learning have maintained their significance in research affiliated with Turkey throughout the years. While COVID-19 studies intensified during the period of 2017-2021, in recent years, the efforts have shifted more toward the healthcare domain (68,69).

CONCLUSION

Our study comprehensively evaluated original and review articles in the field of medical informatics from 1980 to the present using bibliometric methods, focusing on country-, researcher-, institution-, and citation-, and keyword-specific measures and patterns. It was observed that international collaboration in medical informatics research with Turkish affiliation is quite low. Researchers were found to collaborate with their colleagues in prestigious institutions abroad mainly in countries such as the USA, England, Germany, and France. The research, for the most part, revolves around specific universities, with researchers making personal efforts and aligning with prominent figures in the field. Furthermore, the scientific productivity of institutions in the field of medical informatics located in Turkey has been observed to be notably low. Organizing conferences indexed in the CPCI-S would be highly beneficial for these institutions to achieve better standings in international ranking systems. It is recommended that TUBİTAK particularly encourages and supports such conferences. Additionally, it is believed that the establishment of a journal associated with medical informatics research in Turkey, indexed in the SCIE, would positively contribute to the advancement of the field.

Enhancing the scope and quality of education in health and medical informatics will contribute to improving the quality and efficiency of healthcare services in Turkey in the long run. Although the study was conducted in Turkey, it can be argued that the developments in medical informatics research exhibit similarities for all countries, given the universal nature of science. The current and emerging research topics in medical informatics research are expected to influence the curriculum of medical informatics programs in different countries. Particularly, topics like artificial intelligence and machine learning are crucial for healthcare professionals and have been extensively covered in the literature. These subjects have become integral to processes in healthcare services in recent years. Therefore, it is strongly recommended for policymakers to promptly add at least one or two courses related to these topics in the curriculums.

Although our study primarily delves into the broader research landscape within the field of medical informatics in Turkey, it is posited that these overarching trends are likely to align with global patterns. This alignment is attributed to the fact that advancements in scientific research are frequently influenced by contemporary issues. Consequently, the discussions and recommendations presented in this study hold potential utility for researchers across the entire spectrum of medical informatics, particularly those situated in upper-middle and mid-income countries currently engaged in refining their research landscapes.

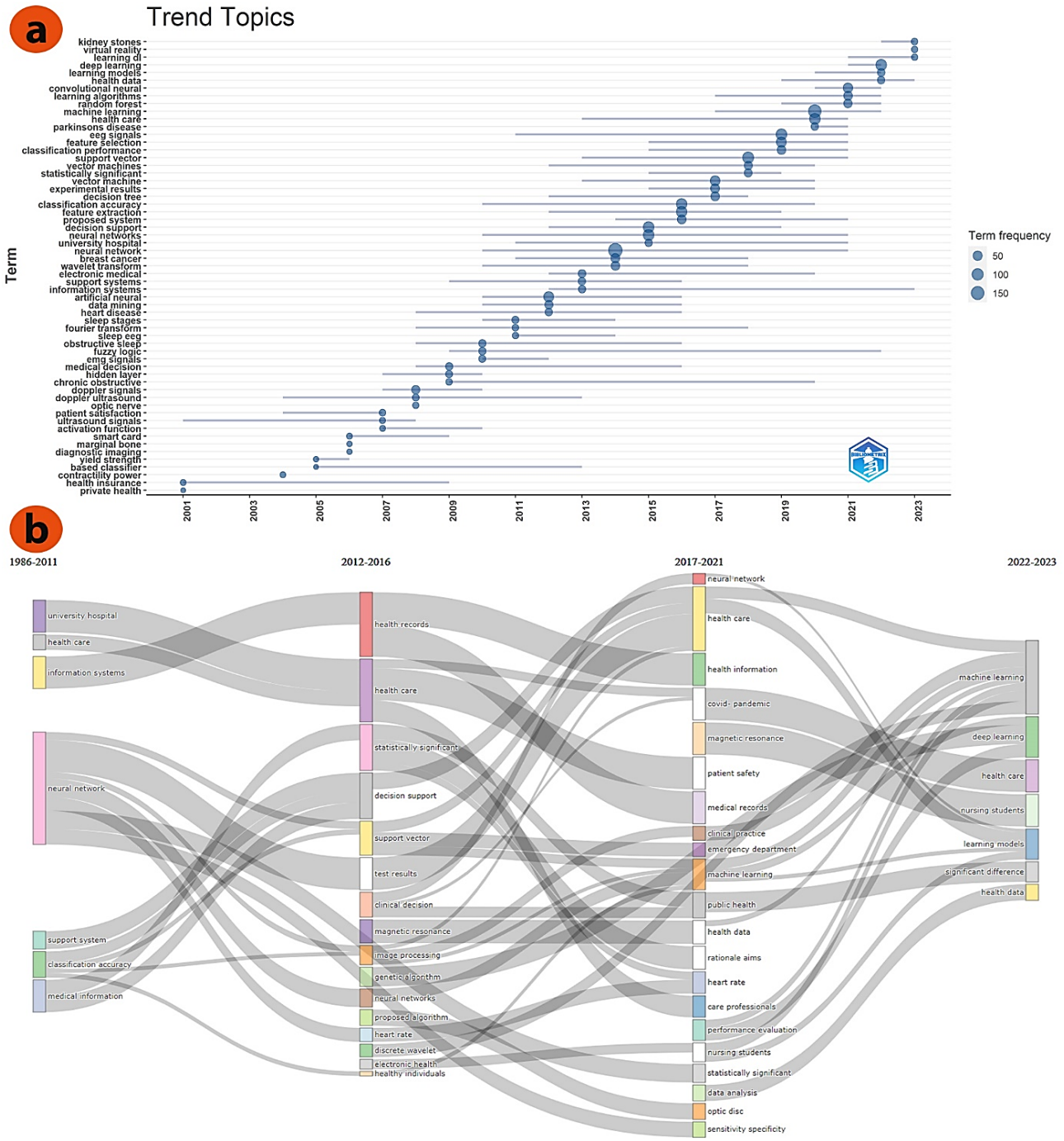

Figure 3. Research words features according to abstract used in the trend topic analysis (a) and thematic evaluation (b)

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Evaluation of Patients with Multiple Sclerosis in Terms of Memory, Attention, Executive Functions, Fine Motor Movement and the Association thereof with Magnetic Resonance Imaging Results

Multiple Sklerozlu Hastaların Bellek, Dikkat, Yürütücü İşlevler, İnce Motor Hareket Yönünden Değerlendirilmesi ve Bunların Manyetik Rezonans Görüntüleme Bulguları ile İlişkisi

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ABSTRACT

Aim: This study aimed to review cognitive function and fine motor skills in patients with multiple sclerosis (MS) and investigate the association with magnetic resonance imaging (MRI) results.
Material and Methods: The study included 22 patients diagnosed with relapsing-remitting MS and 22 controls. Participants underwent neuropsychological tests, including the Stroop test, Rey auditory verbal learning test (RAVLT), line bisection test (LBT), serial reaction time test (SRTT), and finger tapping test (FTT). The relationship between severity of disease, MRI, and test performance was investigated.
Results: It was determined that the patients were lateralized to the right in the LBT data, while the control group was lateralized to the left (p=0.024). On cognitive tests, there was no significant difference in Stroop test results (p=0.134), but the mean overall RAVLT of the patient group was significantly lower than that of the control group (p<0.001). Patients had significantly longer reaction times in SRTT (p=0.038). A positive correlation was found between the expanded disability status scale (EDSS) score and LBT results (r=0.326, p=0.031), and a negative correlation between RAVLT results and EDSS score. For fine motor skills, a negative correlation was observed between FTT results and the number of MS plaques in the left hemisphere (r=-0.431, p=0.045), and a positive correlation between the number of SRTT error and the number of plaques in the juxtacortical regions (r=0.461, p=0.031).
Conclusion: This study shows that the impairment of cognitive functions in MS disease is associated with both the course and severity of the disease and MRI findings.
Keywords: Multiple sclerosis; cognitive functions; neuroanatomical localization; fine motor skills.

ÖZ

Amaç: Bu çalışmada multipl skleroz (MS) hastalarının bilişsel işlevleri ve ince motor becerilerinin gözden geçirilmesi ve bunların manyetik rezonans görüntüleme (MRG) sonuçları ile ilişkisinin araştırılması amaçlandı.
Gereç ve Yöntemler: Çalışmaya relaps-remisyon tipi MS tanısı alan 22 hasta ve 22 kontrol grubu dahil edildi. Katılımcılara Stroop testi, Rey işitsel sözel öğrenme testi (Rey auditory verbal learning test, RAVLT), çizgi bölme testi (ÇBT), seri seçim reaksiyon testi (SSRT) ve parmak vuru testi (PVT) dahil olmak üzere nöropsikolojik testler uygulandı. Hastalık şiddeti ve MRG ile test performansı arasındaki ilişki de incelendi.
Bulgular: Elde edilen sonuçlara göre ÇBT verilerinde hastaların sağa lateralize olduğu, kontrol grubunun ise sola lateralize olduğu tespit edildi (p=0,024). Bilişsel testlerde, Stroop testi sonuçları arasında anlamlı bir fark bulunmazken (p=0,134), hasta grubunun genel RAVLT ortalaması kontrol grubuna göre anlamlı derecede düşüktü (p<0,001). Hastaların SRTT'deki ortalama reaksiyon süresi anlamlı derecede daha uzundu (p=0,038). Genişletilmiş engellilik durumu ölçeği (expanded disability status scale, EDSS) skoru ile ÇBT sonuçları arasında pozitif bir korelasyon (r=0,326; p=0,031) ve RAVLT sonuçları ile EDSS skoru arasında negatif bir korelasyon belirlendi. İnce motor becerilerde, sağ el PVT sonuçları ile sol hemisferdeki MS plağı sayısı arasında negatif bir korelasyon (r=-0,431; p=0,045) ve SSRT hata sayısı ile jukstakortikal bölgelerdeki plak sayısı arasında pozitif bir korelasyon (p=0.031) tespit edildi.
Sonuç: Bu çalışma, MS hastalığında bilişsel işlevlerdeki bozulmanın hem hastalığın seyri ve şiddetiyle hem de MR bulgularıyla ilişkili olduğunu göstermiştir.
Anahtar kelimeler: Multipl skleroz; bilişsel işlevler; nöroanatomik lokalizasyon; ince motor beceri.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic, demyelinating, and degenerative disease of the central nervous system associated with an inflammatory and immune process primarily targeting myelin, which is prevalent in young adults (1). The pathophysiology of MS is a process involving a dynamic interaction of damage and repair mechanisms. The aforementioned interaction is considered to play an important role in determining the clinical course of the disease (2). The regions, where plaques most prevalently occur, include the area around the lateral ventricle (especially between the nucleus caudate and corpus callosum), the floor and roof of the 4th ventricle, optic nerve, pons, around the aqueduct, and medulla spinalis (1).

Demyelination is an acute process and is usually reversible within a few days, yet recovery is associated with remission of the lesion and surrounding edema and acute inflammatory changes. Remyelination is a slow and partial process that can induce functional effects, including slowing of nerve conduction in the central nervous system. MS symptoms may manifest as motor, sensory, visual, cerebellar, sphincter, fatigue, and cognitive disorders associated with demyelination (2,3). Although fatigue and cognitive impairment have been attached less importance compared to other symptoms, they may prove to be the most significant complaint for certain patients (2). Cognitive impairment is a condition that significantly affects the quality of life of patients with MS and is associated with decreased functionality (4). At the same time, it was reported that the decrease in attention and verbal memory performance over time decreases the possibility of finding and retaining a job (5,6). Although all types of cognitive impairment can occur in MS, it is well-established that it mostly involves information processing speed, memory, attention, executive functions, and visuospatial functions (7).

Fine motor movements are defined as movements performed by small muscle groups cooperating in a concerted manner. There is a limited number of comprehensive studies, which investigated slowing in fine motor movement in patients with MS. The results of previous studies reported impairment in fine motor skills in patients with MS (8-11).

The present study aimed to investigate the impairment in cognitive functions and changes in fine motor skills of patients with MS by means of specific tests. It also aimed to investigate the contribution of plaque localization to possible cognitive impairments by correlating test results with magnetic resonance imaging (MRI) results. This work can help researchers better understand the clinical aspects of MS and improve strategies for the management of the disease.

MATERIAL AND METHODS

This study includes patients aged between 18 and 50 years previously diagnosed with relapsing-remitting multiple sclerosis (RRMS) at the Neurology outpatient clinic of Kırıkkale University Faculty of Medicine, with an expanded disability status scale (EDSS) score below 4, and healthy controls matched in age, gender, and educational level. Access to the healthy control group was achieved through random selection from a community-based sample. Participants were informed about the study beforehand and consent forms were obtained.

Participants underwent neuropsychological tests such as the finger tapping test (FTT), serial reaction time test (SRTT), Stroop test, Rey auditory verbal learning test (RAVLT), and line bisection test (LBT), in addition to Beck's depression and anxiety inventories, and hand preference determination tests. The study included a total of 44 participants, comprising 22 RRMS patients and 22 healthy controls. Individuals who had experienced optic neuritis attacks and those with active psychiatric, neurological, visual, or auditory disabilities that could affect test results were excluded. The primary endpoint was the differential performance in neuropsychological tests between MS patients and the control group. The secondary endpoint was the correlation of MS patients' test results with their MRI and EDSS scores. The sample size was determined based on the prevalence of RRMS and the availability of eligible participants during the specified period. This sample size is considered to provide sufficient statistical power to detect clinically significant differences and associations. This study was conducted in accordance with the principles of the Declaration of Helsinki, following the approval from the ethics committee of Kırıkkale University Faculty of Medicine dated February 4th, 2013, and numbered 02/02.

Tests Used in the Study

Stroop Test: This is a three-part cognitive function assessment test developed by J. R. Stroop in 1935. For the first part of the test, the subjects are provided with color names and asked to read them as fast as possible. For the second part, the subjects are asked to say the colors of the dot clusters printed in colored ink as fast as possible; whereas for the third part, the subjects are asked to read the words written in ink of a different color than the name of the presented color as fast and loudly as possible. The importance of this test in terms of cognitive assessment is based on the fact that visual perception predominates when there is a conflict between visual perception and symbolic-semantic perception (12). The Stroop test is a neuropsychological frontal region test used to assess functional impairments associated with brain injury. It is generally believed that the Stroop test measures the ability to resist interference (the ability to maintain attention despite interference).

Rey Auditory Verbal Learning Test (RAVLT): The original form of the RAVLT comprised of word lists was developed by Rey in 1964. The RAVLT is a multi-aspect test of information processing of verbal material. These processes include verbal learning, immediate memory span, retroactive interference, free recall, and recognition memory. This test can make a quantitative assessment of both memory-related parameters and parameters determined by experimental psychological studies (13). The importance of instruments such as the RAVLT is based on the fact that they allow for a valid assessment of memory functioning.

Line Bisection Test (LBT): LBT is a test used to investigate neglect in neglect syndrome that is associated with brain injuries and "pseudoneglect" defined as neglect of the right hemisphere in healthy individuals (14). The LBT used in this study was developed by Nalçacı et al (15).

The test-retest reliability of the hardcopy form of the test was investigated in young adults by Güneş et al. (14). The mean both hand results of the tested subjects indicated that the lines presented in the left field were divided significantly to the left of the midpoint. This is called as physiological neglect. In the present study, papers with 8 cm, 14 cm, and 20 cm lines, including four lines each, were given and the participants were asked to make a mark that would divide these lines exactly in the middle. Subsequently, the average of the length group was taken separately and the deviation to the right or left (deviation in the left direction was negative, deviation in the right direction was positive) and the distance away from the center were calculated for each group, and the resultant data were statistically evaluated.

Serial Reaction Time Test (SRTT): Frequently used as a simple reaction test in studies, especially in the last 20 years, SRTT is a test characterized by responding appropriately to a stimulus after a stimulus is presented on a computer screen. The stimulus appears on the computer screen for varied durations. In the present study, the time between stimuli varied between 1 and 2 seconds. Therefore, by preventing the individual's temporal adaptation to the arrival of stimuli, it was aimed to make the preparation of the individual for the stimulus and direct attention dominant. The stimulus appears to the right or left of the focal point on the display. Depending on the side the stimulus appears, it is asked to press the button of the mouse on that side as quickly as possible. The next stimulus appears after the individual presses the button. Therefore, this test measures both the attention by capturing the appropriate response to the stimulus and the response time to the stimulus and therefore, the visuomotor performance (16).

Finger Tapping Test (FTT): FTT is a test that has been used since the 19th century aimed to assess fine motor performance. It was reported to be correlated with high levels of intelligence and high neuropsychological test scores, and it is a characteristic test, especially in assessing the motor performance of the upper extremity (17). In the present study, the computer mouse was used to repeatedly hit the right and left buttons as fast as possible for 15 seconds. The FTT value was determined as the number of button hits in 15 seconds.

Statistical Analysis

Repeated measures analysis of variance was conducted, where the amount of deviation in millimeters in the LBT was taken as the dependent variable, and line length and group were taken as independent variables. Considering the number of taps in the FTT as the dependent variable, a repeated measures analysis of variance was conducted for hand and group. Assumptions of normality were verified using the Shapiro-Wilk test. Test scores of the groups were compared by t-test. EDSS values and test scores were evaluated with Pearson correlation analysis to assess the correlation between severity of disease and test performances. The number of MS plaques and test scores were evaluated by Pearson correlation analysis to assess the correlation between MRI results and test performance. All statistical assessments were conducted using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA) software, with two-tailed tests and a p-value of <0.05 considered statistically significant.

RESULTS

The study involved 22 (15 females and 7 males) MS patients and a matched control group of 22 (15 females and 7 males) healthy individuals, with no significant differences in age, gender, and educational level. The mean age of the MS group was 32.5±6.6 years, while the mean age was 32.0±6.6 years in the control group. The dependent variable for the groups in terms of their LBT performance was taken as the deviation in millimeters from the center. Accordingly, the group main effect was investigated ($f_{(1,4)}$=5.515, p=0.024). The results suggested that the patient group exhibited right lateralization, whereas the control group showed left lateralization. In the LBT measurements, it was observed that the patient group exhibited average deviations of 0.018, 0.091, and 0.468 mm for LBT 8, 14, and 20 cm, respectively, while the control group showed -0.318, -0.555, and -0.791 mm for the same measurements. There was no significant difference in the main effect of line length and group effect of line length (p=0.696, p=0.053).

FTT was conducted twice using both hands. Aside from the main effects for hand and group (p<0.001, p=0.002), no significant effects or interactions were noted. The mean FTT measurements were 72.77±15.76 and 86.46±10.49 for right1, in patient and control groups, respectively. The means were 73.96±19.09 and 85.36±10.66 for right2, 60.59±16.48 and 75.18±7.69 for left1, and 62.68±15.70 and 73.32±8.82 for left2.

The results of the Stroop test were analyzed upon calculating the Stroop interference. The analysis results showed that the average Stroop interference for the patient group was 1.067, while for the control group, it was 3.867. These findings indicate that there was no significant difference in Stroop interference between the patient and control groups (p=0.134). The SRTT reaction time results were suggestive of the fact that the reaction time in the patient group was significantly longer compared to that of the control group (p=0.038). The SRTT error results showed that there was no significant difference in the number of errors between the patient group and the control group (p=0.336). Based on the RAVLT results, there was a significant difference between the two groups in all 9 steps of the test, and the patient group could have recalled fewer words in each step (Table 1).

Table 1. Comparison of the SRTT and RAVLT results

	Patient (n=22)	Control (n=22)	p
SRTT	40.75±14.18	33.42±7.51	**0.038**
SRTT error	1.05±1.21	0.73±0.94	0.336
RAVLT 1	5.64±1.62	7.64±1.59	**<0.001**
RAVLT 2	7.68±1.91	11.00±1.83	**<0.001**
RAVLT 3	9.32±2.46	12.18±1.68	**<0.001**
RAVLT 4	10.36±2.46	13.45±1.30	**<0.001**
RAVLT 5	10.86±2.42	14.64±0.58	**<0.001**
RAVLT 6	5.18±1.59	6.36±1.36	**0.011**
RAVLT 7	8.27±2.47	13.32±1.36	**<0.001**
RAVLT 8	8.32±2.70	13.73±0.88	**<0.001**
RAVLT recognition	9.36±3.42	14.50±0.86	**<0.001**

SRTT: serial reaction time test, RAVLT: Rey auditory verbal learning test

EDSS scores and test performance results were assessed with Pearson correlation analysis to see the effect of the severity of MS disease on test performance. There was a positive correlation between the 20 cm LBT test and EDSS scores (r=0.326, p=0.031). As the EDSS score increased, the deviation from the center increased. Upon review of EDSS and FTT results, there was a negative correlation between EDSS and FTT performance. As the EDSS score increased, FTT performance decreased. There was no significant correlation between EDSS and Stroop test scores. While there was a positive correlation between EDSS and SRTT reaction time (r=0.359, p=0.017), there was no significant correlation between EDSS and SRTT error rate (r=0.117, p=0.450). An analysis of the EDSS and RAVLT correlation showed a negative correlation for all subtest results of RAVLT (Table 2).

MRI findings in MS patients were analyzed to determine the smallest lesion diameter (SLD) and largest lesion diameter (LLD). The mean SLD was 3.8±0.3 mm, and the mean LLD was 17.0±1.3 mm across all patients.

There was no significant correlation between the number of MS plaques and LBT. Upon review of the periventricular, juxtacortical, and infratentorial regions of the hemisphere (contralateral), which ensured motor control of the hand, the right-hand FTT performance decreased as the number of MS plaques in the left juxtacortical region increased (r=-0.431, p=0.045). There was no significant correlation between FTT left-hand performances and the number of MS plaques in the right hemisphere (Table 3). No significant correlation was found between Stroop interference and the number of MS plaques. While there was no significant correlation between SRTT reaction time and the number of MS plaques, there was a positive correlation between the SRTT errors and juxtacortical right and juxtacortical left (Table 4). Upon review of the subtests of the RAVLT, which assessed language skills in a very broad framework, the number of plaques in the juxtacortical region was mostly associated with RAVLT scores (Table 5). Besides, the number of plaques in the left infratentorial region was also correlated with the RAVLT subtests (Table 6).

DISCUSSION

Cognitive impairment is a condition that is associated with significant impairment of the quality of life of patients with MS. Physical independence, ability to perform daily activities, symptom management, coping, treatment adherence, and rehabilitation are particularly affected subgroups. Fine motor movements, which are important for performing daily activities are also affected by MS. The study results showed impairment in all the tests aimed to assess cognitive functions. Furthermore, the deterioration in their test performance became more manifest upon increasing severity of MS.

Table 2. Correlation of EDDS and test performance results

	EDDS	
	r	**p**
LBT 8 cm	0.129	0.403
LBT 14 cm	0.255	0.095
LBT 20 cm	0.326	**0.031**
FTT right 1	-0.578	**<0.001**
FTT right 2	-0.487	**0.001**
FTT left 1	-0.612	**<0.001**
FTT left 2	-0.475	**<0.001**
SRTT	0.359	**0.017**
SRTT error	0.117	0.450
RAVLT 1	-0.517	**<0.001**
RAVLT 2	-0.600	**<0.001**
RAVLT 3	-0.558	**<0.001**
RAVLT 4	-0.605	**0.042**
RAVLT 5	-0.732	**<0.001**
RAVLT 6	-0.308	**<0.001**
RAVLT 7	-0.708	**<0.001**
RAVLT 8	-0.768	**<0.001**
RAVLT recognition	-0.662	**<0.001**

EDSS: expanded disability status scale, LBT: line bisection test, FTT: finger tapping test, SRTT: serial reaction time test, RAVLT: Rey auditory verbal learning test

Table 3. Correlation of FTT right-hand performance and left hemisphere MS plaque count

	FTT right 1		FTT right 2	
Left	**r**	**p**	**r**	**p**
Periventricular	-0.040	0.858	0.033	0.884
Juxtacortical	-0.396	0.068	-0.431	**0.045**
Infratentorial	-0.205	0.360	-0.126	0.575

FTT: finger tapping test, MS: multiple sclerosis

Table 4. Correlation of the number of SRTT error and the number of MS plaques

	SRTT Error	
	r	**p**
Periventricular right	0.244	0.273
Periventricular left	0.167	0.457
Juxtacortical right	0.443	**0.039**
Juxtacortical left	0.461	**0.031**
Infratentorial right	0.098	0.666
Infratentorial left	0.206	0.358

SRTT: serial reaction time test, MS: multiple sclerosis

Table 5. Correlation analysis between RAVLT subtests and juxtacortical right and juxtacortical left MS plaque count

	RAVLT 4		RAVLT 5		RAVLT 7		RAVLT 8		RAVLT recognition	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Juxtacortical										
Right	-0.460	**0.031**	-0.495	**0.019**	-0.466	**0.029**	-0.615	**0.002**	-0.521	**0.013**
Left	-0.348	0.112	-0.408	0.059	-0.400	0.065	-0.511	**0.015**	-0.522	**0.013**

RAVLT: Rey auditory verbal learning test, MS: multiple sclerosis

Table 6. Correlation analysis between RAVLT subtests and infratentorial left MS plaque count

	RAVLT 3		RAVLT 4		RAVLT 8	
	r	**p**	**r**	**p**	**r**	**p**
Infratentorial left	-0.462	**0.030**	-0.470	**0.027**	-0.454	**0.034**

RAVLT: Rey auditory verbal learning test, MS: multiple sclerosis

The SRTT test showed a significant increase in reaction time in patients with MS. The increase in cognitive load with increasing difficulty of the task was indicative of the fact that this test was effective in assessing cognitive processing speed. Moreover, there was a positive correlation between EDSS scores and SRTT reaction times, suggestive of the fact that as the severity of the disease increased, the information processing time was prolonged. Upon error analysis, patients with MS had longer reaction times, though the number of errors was similar to the control group. This suggested that the prolongation of reaction time might be a result of an effort to reduce the rate of error. Previous studies also supported that SRTT results indicated longer reaction times in patients with MS and that this difference became further manifest with increased severity of disease (18-22).

Previous studies on memory in patients with MS generally reported impaired memory (23-29). Miyazaki et al. (30) investigated the cognitive functions of patients with MS using the symbol digit modalities test (SDMT), California verbal learning test-second edition (CVLT-2), and brief visuospatial memory test-revised (BVMT-R). The above study investigated associations between clinical characteristics, patterns of brain volume loss, and cognitive test results. SDMT performance declined through the course of MS disease in association with brain volume loss. It was concluded that BVMT-R performance also decreased in parallel with brain volume loss, but the deterioration in CVLT-2 became more manifest, especially during the later stages of MS (30). In the present study, patients with MS had impaired memory performance in all subtests of the RAVLT. The patients with MS remembered significantly fewer words compared to the control group.

In addition, as a result of the analyses in the scope of the study, there was a negative correlation between EDSS scores and RAVLT performance. This result suggested that as the severity of the disease increased, the number of recalled words decreased. Notwithstanding the above, there was a significant negative correlation between RAVLT subtest scores and left infratentorial region, right, and left juxtacortical region, and the number of plaques. This result suggested that damage to those brain regions was associated with impaired memory performance. Various studies investigated the cognitive functions of patients with MS using different tests. In a study to evaluate the cognitive functions in patients with MS, Scherer et al. (24) reported that patients had lower performance in the digit symbol substitution test (DSST), paced auditory serial addition test (PASAT), and faces symbol test (FST). Schulz et al. (26) reported impaired processing speed and memory problems in patients with MS. Demers et al. (28) found significant differences in patients with MS, but reported that there was no linear relationship between cognitive impairment and severity of disease. Cerezo Garcia et al. (25) reported impairment in executive functions, while Hoffman et al. (31) found impairment in working memory in most participants. Sehanovic et al. (32) showed that 40-60% of patients with MS had impaired cognitive functions and that this effect became more manifest with an increase in disease duration. Consistent with the above studies, the results of the present study indicated that memory impairment in patients with MS was positively associated with disease duration, and also with disease severity. For the purposes of the present study, participants without depressive disorders were included in both the MS patient group and the control group to exclude the effect of depression on cognitive functions. Previous studies suggested that individuals with MS had impairment in complex attention tasks. This was usually associated with working memory and executive attention (33). Attention is maintained by an important neural network widely distributed across the brain. These networks include regions such as the prefrontal cortex, parietal cortex, and cingulate gyrus, which are tightly interconnected. It has been emphasized that these regions are critical in various tasks varying between motor executive functions and information encoding and planning and executive functions of attention (34,35). In the present study, there was no significant difference between the groups by Stroop test performance. This was suggestive of the fact that patients with MS might have memory impairment independent of executive functions. Furthermore, there was no significant difference between the groups upon analysis of the number of errors component of the SRTT. Nevertheless, there was a positive correlation between the number of SRTT errors and the number of MS lesions in the left and right juxtacortical regions. In other words, the participants with a higher number of SRTT errors had a higher number of MS lesions in these regions. It was likely that the above regions were located at the connections between regions critical for attentional function.

De Sonneville et al. (36) reported that patients with MS had significant impairments in all attention domains. A study by Bodling et al. (18) suggested a remarkable slowing of response time and inconsistencies and errors between the responses in patients with MS. The authors suggested that the foregoing prolongation in response time in MS primarily indicated a slowdown in the speed of information processing, but there was also an attentional impairment. In the present study, the fact that the SRTT reaction time was longer and there was no difference in the number of errors in the Stroop test and SRTT in patients with MS, indicated that there was an impairment in the information processing process rather than attention.

It was seen that the left hemisphere lesions tended not to affect spatial attention, but right hemisphere lesions might have affected attention directed to left space. This condition is known as the "neglect phenomenon" and

affects the spatial awareness of the patients. Patients with neglect phenomena can focus only on the right space in visual drawings or written texts while neglecting the left space. For example, they can copy only the right side of a drawing or read only the right half of a text. A patient with left space neglect may divide the lines closer to the right, considering that they would divide the lines in the center, and as a result may leave a longer space on the left compared to the right (37). A study by Kocsis et al. (38) reported the left-sided tendency of pseudoneglect in healthy individuals, whereas it was concluded that lesions affecting the integrity of white matter pathways in patients with MS might increase the variability of spatial attentional bias. In a study by Gilad et al. (39), LBT, a random shape cancellation test was used and its correlation with brain MRI was investigated. Based on the results of LBT, significant right lateralization was observed in the MS patient group compared to the healthy controls. Furthermore, the results of the random cancellation test indicated high error rates on the left side in patients with MS. Nevertheless, there was no significant correlation upon the comparison of the test results with MRI results. In the present study, although all the participants were right-handed, participants in the control group lateralized to the left, whereas patients with MS lateralized to the right. In addition, the deviation from the center was significantly higher as the line length increased in the MS patient group. Patients with MS were significantly more distanced from the center in the 20 cm subtest of the LBT. There was a positive correlation between the LBT 20 cm test and EDSS; i.e., the deviation from the center increased with increasing disease severity in the longer lines. This suggested that although patients with MS could compensate for spatial attentional impairment in short lines and/or narrow space, the compensation mechanism was not sufficient and the impairment became more pronounced as the line got longer/space widened. Nevertheless, the results of the study indicated that there was no correlation between the number of MS plaques in any region and the performance of LBT. Therefore, the LBT data suggested that the spatial distribution of patients' attention differed from the controls (that negligence changed direction) and that disease severity made the foregoing difference more pronounced as line length increased.

Fine motor movements are the movements performed upon coordinated work of small muscle groups. A previous study reported that the primary motor area (M1) was the general main control center for simple voluntary movements and the premotor area played an auxiliary role in those movements (40). There are only a limited number of studies investigated slowing in fine motor movements in MS. Nevertheless, a study by Longstaff et al. (8) reported that patients with MS performed the task of drawing a spiral on a graphic tablet more slowly, applying less pressure, and deviating more from the ideal trajectory compared to the control group. These results were interpreted as an indicator of fine motor movement impairment in patients with MS. In the present study, there were significant differences between the groups by both FTT performance, a pure motor test, and SRTT performance, which reflected the cognitive component of fine motor movement. Patients with MS were slower, had longer SRTT reaction times, and had fewer FTT taps compared to the healthy controls. Based on these results, it was suggested that patients with MS had impaired fine motor movement performance and that this impairment was induced by both cognitive and motor processes. Besides, there was a negative correlation between EDSS scores and FTT performance and a positive correlation between SRTT reaction time and EDSS scores. In other words, as the severity of the disease increased, the number of FTT taps decreased and the SRTT reaction time increased. Therefore, it can be suggested that impairment in fine motor skills becomes more manifest as the severity of MS disease increases. Furthermore, the correlation between FTT performance and the number of MS plaques in the periventricular, juxtacortical, and infratentorial regions of the hemisphere (contralateral), which provided motor control of the hand, suggested that right-hand FTT performance decreased as the number of MS plaques in the left juxtacortical region increased. Nevertheless, there was no significant correlation between the left-hand FTT performance and the number of plaques in the right hemisphere. This result is suggestive of the fact that the motor problem due to MS plaques became more pronounced as the functional burden/responsibility became more apparent.

The results of the present study indicated impairment in cognitive domains, including spatial orientation of attention, information processing time, and verbal memory. It also suggested that fine motor skills were affected in both cognitive and motor domains. The significant correlations observed between MRI results and these impaired functions provided insight into the neuroanatomical localization of cognitive functions. The severity of the disease and the localization of the MS plaque had an impact on the prominence of this impairment. Accordingly, functional MRI or event-related monitoring during testing may not only provide information about the neuroanatomical localization of both cognitive functions and fine motor skills but also information about cognitive impairment during the disease. These results provided significant clues to better understand the effects of MS on cognitive functioning and the relationship between the severity of the disease and these effects. Nevertheless, it should be noted that further studies can help deepen our understanding of this issue.

Limitations of this study include the relatively small sample size, which may limit the generalizability of the findings, the cross-sectional design, which limits the ability to determine long-term effects, and the fact that the sample only represents a specific subgroup of MS patients. Additionally, the tests used and the MRI findings have their own limitations, which should be considered when interpreting the results.

CONCLUSION

The results indicative of the fact that the impairment in cognitive functions of patients with MS was directly proportional to the course and severity of the disease, may shed light on future studies in this field. Furthermore, the MRI results in the present study provided a more comprehensive perspective by helping us better understand the association between these cognitive impairments and neuroanatomical localization.

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The Role of Beta hCG Value Measured on the 12th and 14th Days After Embryo Transfer in Determining Early Complications of Pregnancy

Embriyo Transferi Sonrası 12. ve 14. Günlerde Ölçülen Beta hCG Değerinin Gebelikte Erken Komplikasyonların Belirlenmesindeki Rolü

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ABSTRACT

Aim: This study aimed to investigate the role of beta human chorionic gonadotropin (beta hCG) levels on post embryo transfer (ET) 12th- and 14th-day, and its folding after 48 hours in predicting live birth, abortion, and biochemical pregnancy.
Material and Methods: The study included 124 patients who had a positive pregnancy test after a fresh single day 3 ET at the in vitro fertilization (IVF) center between 2017 and 2021. The first beta hCG value was measured 12th day and the second 14th day after ET. The beta hCG fold was calculated by dividing the second beta hCG value by the first beta hCG value.
Results: The patients' IVF indications included unexplained (n=40, 41.1%), poor ovarian reserve (n=23, 25.0%), male factor (n=31, 29.8%), and tubal factor (n=3, 4.1%). Of the 124 patients, 97 (78.2%) had a fetal sac, 81 (63.5%) had a fetal heartbeat (FHB), and 70 (56.5%) had a live birth. The results indicated that the post-ET 14th-day beta hCG level was the best predictor of biochemical pregnancy. It has a high sensitivity (92.5%) and specificity (86.6%), with an optimal cut-off value of 175 U/L. The post-ET 14th-day beta hCG level was the best predictor of a live birth. The post-ET 14th-day beta hCG value of 214.5 U/L had an 82.7% sensitivity and 74.4% specificity to predict the FHB.
Conclusion: The beta hCG value, measured between the 12th and 14th days after ET, as well as the folding rate on these two days, can provide information about the pregnancy progression.
Keywords: Beta hCG; live birth rate; post embryo transfer; pregnancy outcome.

ÖZ

Amaç: Bu çalışmanın amacı, embriyo transferi (ET) sonrası 12. ve 14. gündeki beta human chorionic gonadotropin (beta hCG) düzeylerinin ve 48 saat sonraki katlanmasının canlı doğum, düşük ve biyokimyasal gebeliği öngörmedeki rolünün araştırılmasıdır.
Gereç ve Yöntemler: Çalışmaya 2017 ve 2021 yılları arasında tüp bebek (in vitro fertilization, IVF) merkezinde taze tek 3. gün ET sonrası gebelik testi pozitif çıkan 124 hasta dahil edildi. İlk beta hCG değeri ET'den sonra 12. gün, ikincisi ise 14. gün ölçüldü. Beta hCG katlanması, ikinci beta hCG değerinin birinci beta hCG değerine bölünmesiyle hesaplandı.
Bulgular: Hastaların IVF endikasyonları arasında açıklanamayan (n=40, %41,1), kötü over rezervi (n=23, %25,0), erkek faktörü (n=31, %29,8) ve tubal faktör (n=3, %4,1) yer alıyordu. 124 hastanın 97'sinde (%78,2) gebelik kesesi, 81'inde (%63,5) fetal kalp atışı (fetal heartbeat, FHB) ve 70'inde (%56,5) canlı doğum gerçekleşti. Sonuçlar, ET sonrası 14. gün beta hCG düzeyinin biyokimyasal gebeliğin en iyi belirleyicisi olduğunu gösterdi. 175 U/L optimum kesim değeri ile yüksek bir duyarlılığa (%92,5) ve özgüllüğe (%86,6) sahipti. ET sonrası 14. gün beta hCG düzeyi canlı doğumun da en iyi belirleyicisiydi. ET sonrası 14. gün 214,5 U/L beta hCG değerinin FHB'yi öngörmedeki duyarlılığı %82,7, özgüllüğü ise %74,4 idi.
Sonuç: ET sonrası 12. ve 14. günler arasında ölçülen beta hCG değeri ve bu iki gündeki katlanma oranı gebeliğin seyri hakkında bilgi verebilir.
Anahtar kelimeler: Beta hCG; canlı doğum oranı; embriyo transferi sonrası; gebelik sonucu.

INTRODUCTION

Beta human chorionic gonadotropin (beta hCG) released by trophoblasts is detected in the blood 6-8 days after fertilization. It has been shown that 12-16 days after in vitro fertilization (IVF) treatment, its level is predictive of pregnancy outcome. In general, elevated initial serum beta hCG levels indicate a good prognosis (1).

Approximately 22% of IVF pregnancies result in miscarriage. Patients undergoing IVF experience intense anxiety and stress during their first pregnancy test. Identifying an accurate predictor of pregnancy following embryo transfer (ET) can reduce patient stress (2). Many studies based on the fetal yolk sac, which is one of the fetal transvaginal ultrasonography findings, as well as the size of the fetal sac and the doubling of the beta hCG test, have made significant contributions to the literature in determining pregnancy outcomes. For example, Deaton et al. (3) discovered that the presence of a yolk sac between 22 and 32 days after ET indicated fetal cardiac activity development in 94% of patients, whereas its absence was associated with 100% spontaneous abortion. However, none of these ultrasonographic findings can be detected before the fifth or sixth week of pregnancy. There are a few studies (4-6) looking into the relationship between the initial beta hCG result and live birth, and there is no ideal cut-off value for clinical and live birth predictions.

In this study, we aimed to investigate the role of post-ET 12th- and 14th-day beta hCG levels and their fold in two days to predict early pregnancy outcomes by determining possible cut-off values.

MATERIAL AND METHODS

After approval from the University of Health Sciences Hamidiye Scientific Research Ethics Committee, İstanbul on 08.04.2022 date and 22/209 number, medical records are retrospectively collected from patient files. The study only included patients with positive IVF results and excluded multiple pregnancies and ETs due to elevated beta hCG levels, as well as ectopic and heterotropic pregnancies.

A retrospective study was conducted on 124 patients, who tested positive for pregnancy after a fresh single day 3 ET at the IVF center of Dr. Ali Kemal Belviranlı Obstetrics and Gynecology Hospital, Konya, between 2017 and 2021. The first beta hCG measurement was taken on the 12 days after ET, followed by a second measurement two days later. Patients with beta hCG levels greater than 5 U/L are considered to have a positive pregnancy. The same hospital's Medical Biochemistry Laboratory performed beta hCG measurements using an immunometric sandwich assay with the Immulite 2000 system (Siemens Medical Solutions Diagnostics, Flanders, NJ). The assay's sensitivity was 0.4 mIU/mL. The increase in the beta hCG measurement was observed after 48 hours and control was requested to examine the gestational sac (GS) in response to this increase. Patients with GS were invited to check the fetal heartbeat (FHB) 10 days later. Those with a visible FHB on ultrasound were considered clinically pregnant; babies born before 20 weeks and weighing less than 500 g were recorded as abortions; and babies born after 24 weeks were recorded as live births. Biochemical pregnancy is defined as a decrease in beta hCG levels without the presence of an intrauterine or extrauterine GS (7).

The patients' demographic data included age, body mass index (BMI), basal follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) levels, infertility duration, and cause of infertility were recorded. While the short antagonist protocol was initiated for 114 patients, the files revealed that the long agonist protocol was initiated for 10 patients.

In the short antagonist protocol, medication was started on the second day of menstruation. Recombinant FSH (Gonal F, Merck Serono, Italy) or urinary human menopausal gonadotropin (Menogon, Ferring, Germany) doses were determined individually based on the patient's BMI and the causes of infertility. When the follicle diameter reached 13-14 mm, an antagonist (Cetrorelix, Merck Serono, Germany) injection was administered flexibly. In the long protocol, a gonadotropin releasing hormone (GnRH) analog was started during the previous cycle's mid-luteal period (usually the 21st day), and gonadotropins were added after pituitary-ovarian suppression (usually 10 days after agonist use). Both drugs were administered concurrently until the day of the hCG injection. When an optimal cohort of large antral follicles is observed on ultrasound (at least three follicles >18 mm), ovulation is triggered by a single hCG injection (Ovitrelle, Merck Serono). Oocytes were collected after 34-36 hours of hCG injection. All patients underwent the intracytoplasmic sperm injection (ICSI) procedure. To provide luteal phase support, the patients received 600 mg/day of intravaginal progesterone capsules (Progestan 200 mg soft capsule, Koçak Farma, Istanbul). When the pregnancy was confirmed by a positive beta hCG test (>5 U/L), luteal phase support was maintained until the 10th gestation week.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.). Kolmogorov-Smirnov and Shapiro-Wilk tests and histograms were used to determine whether the distribution of continuous variables was normal. Mean and standard deviation values were reported for normally distributed variables. For non-normally distributed variables, median, minimum, and maximum values were presented. The independent sample t-test was used to assess differences between two groups for normally distributed continuous variables, and the Mann-Whitney U test was used for continuous variables that did not show a normal distribution. In the analyses of categorical variables, the chi-square test was used to evaluate the relationships along with number and percentage values. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal threshold values of hCG levels used to predict biochemical pregnancy, positive FHB, and live birth. A two-way p-value of 0.05 was taken as a significance level.

RESULTS

The study included 124 patients who underwent IVF and had positive beta hCG values. The mean age of the patients was 29.7±5.3 (range, 20-43) years. The IVF indications of the patients were unexplained (n=40, 41.1%), poor ovarian reserve (n=23, 25.0%), male factor (n=31, 29.8%), and tubal factor (n=3, 4.1%). Patients have beta hCG values measured for the first time 12 days after ET and for the

second time 2 days after the first test. Of 124 patients included, 97 (78.2%) had a fetal sac, 81 (63.5%) had a FHB, and 70 (56.5%) had a live birth (Table 1).

There were no significant differences between the two groups of GS positive and GS negative in terms of basic demographic and clinical parameters such as age, BMI, FSH, LH, and E2. There were also no significant differences in infertility duration (p=0.161) and IVF indications (p=0.584) between the two groups. The first and second beta hCG values, as well as their ratio, were significantly higher in the GS positive group (p=0.001). The GS positive group had significantly higher positive fetal heart activity at 82.5% (n=80) compared to the rate of 3.7% (n=1) in the GS negative group (p=0.001). The live birth rate was 72.2% (n=70) out of the 97 GS positive patients (Table 2).

When assessed according to the presence of FHB, the FHB positive group showed significant differences in first and second beta hCG levels (193.2 U/L vs 51.7 U/L, p=0.001 and 567.8 U/L vs 141.2 U/L, p=0.001, respectively) and second to first beta hCG ratio (2.67 vs 2.05, p=0.003) compared to the FHB negative group. However, a small but statistically significant difference was also found in BMI (24 kg/m^2 vs 25 kg/m^2, p=0.010). No significant difference was found between FHB positive and negative groups in terms of age, FSH, LH, E2 values, and duration of infertility (Table 3).

Age, FSH, LH, and E2 were similar in the labor and abortion groups. BMI (23.5 kg/m^2 vs 24 kg/m^2, p=0.035) and duration of infertility (5 years vs 6 years, p=0.026) were significantly lower in the labor group than in the abortion group. The first and second beta hCG measurements were significantly higher in pregnancies that ended in labor compared to those that ended in miscarriage (both p<0.001). In addition, the beta hCG ratio was higher in labor than in miscarriage (2.65 vs 2.29, p=0.005, Table 4).

The performance of beta hCG level to predict biochemical pregnancy, FHB positivity, and live birth outcomes following ET was presented in Table 5. It reveals that for the detection of biochemical pregnancy, the beta hCG level measured on post-ET day 14 offers the highest predictive accuracy. A cut-off value of 175 U/L provided a sensitivity of 92.5% and a specificity of 86.6% with an area under the curve (AUC) of 0.952 (95% CI, 0.910-0.993, p=0.001). The second to first beta hCG ratio and beta hCG level on post-ET day 12 also showed significant predictive value, but the highest AUC value and sensitivity were obtained for the day 14 measurement. In the assessment of a positive FHB, which is an important early marker of viable pregnancy, again the beta hCG measurement on post-ET day 14 proved to be highly predictive. A cut-off value of 214.5 U/L was associated with a sensitivity of 82.7%, a specificity of 74.4%, and an AUC of 0.837 (95% CI, 0.761-0.914, p=0.001). The beta hCG ratio and beta hCG

Table 1. Demographic and clinical characteristics of the patients

Age (year)	29 (8) [20-43]
BMI (kg/m^2)	24 (2) [20-29]
Duration of infertility (year)	5 (5) [1-23]
FSH (U/L)	7 (4) [4-16]
LH (U/L)	9 (5) [3-15]
E2 (ng/L)	45 (20) [15-88]
Post-ET 12th-day beta hCG (U/L)	144.3 (208.7) [9.3-880.1]
Post ET 14th-day beta hCG (U/L)	392.5 (550.7) [0.9-2213.5]
Beta hCG ratio	2.55 (1.15) [0.07-9.77]
GS Positivity, n (%)	97 (78.2)
Clinical pregnancy, n (%)	81 (65.3)
Pregnancy outcomes, n (%)	
Chemical pregnancy	26 (21.0)
Abortion	28 (22.6)
Live birth	70 (56.5)

BMI: body mass index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, ET: embryo transfer, hCG: human chorionic gonadotropin, GS: gestational sac, descriptive statistics for continuous variables were presented as median (interquartile range, 75th-25th percentile) [minimum-maximum]

Table 2. Comparison of clinical and demographic characteristics of IVF treatments according to the GS status

	GS positive (n=97)	GS negative(n=27)	p
Age (year)	29 (8) [20-43]	31 (9) [21-42]	0.100
BMI (kg/m^2)	24 (2) [20-29]	24 (3) [21-29]	0.193
Duration of infertility (year)	5 (4) [1-23]	6 (6) [1-15]	0.161
FSH (U/L)	7 (3) [4-15]	8 (6) [4-16]	0.133
LH (U/L)	9 (5) [3-15]	9 (6) [4-15]	0.626
E2 (ng/L)	45 (15.5) [15-88]	45 (24) [15-88]	0.887
Post-ET 12th-day beta hCG (U/L)	189.6 (226.1) [27.9-880.1]	33.5 (47.8) [9.3-175.5]	**0.001**
Post ET 14th-day beta hCG (U/L)	514.0 (495.8) [80.4-2213.5]	48.6 (131.0) [0.9-486.8]	**0.001**
Beta hCG ratio	2.70 (0.92) [1.63-9.77]	1.08 (1.59) [0.07-4.52]	**0.001**
Fetal cardiac activity, n (%)	80 (82.5)	1 (3.7)	**0.001**
IVF indication, n (%)			
Unexplained	40 (41.2)	11 (40.7)	0.584
Poor ovarian reserve	23 (23.7)	8 (29.6)	
Male factor	31 (32.0)	6 (22.2)	
Tubal factor	3 (3.1)	2 (7.4)	
Pregnancy outcome, n (%)			
Miscarriage	27 (27.8)		
Live birth	70 (72.2)		

BMI: body mass index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, ET: embryo transfer, hCG: human chorionic gonadotropin, GS: gestational sac, IVF: in vitro fertilization, descriptive statistics for continuous variables were presented as median (interquartile range, 75th-25th percentile) [minimum-maximum]

Table 3. Comparison of clinical and demographic characteristics according to the status of FHB

	FHB positive (n=81)	FHB negative (n=43)	p
Age (year)	29 (8) [20-43]	300 (10) [21-42]	0.575
BMI (kg/m^2)	24 (2.5) [20-28]	25 (3) [21-29]	**0.010**
Duration of infertility (year)	5 (3.5) [1-23]	6 (6) [1-16]	0.108
FSH (U/L)	7 (3) [4-15]	7 (5) [4-16]	0.769
LH (U/L)	9 (5) [4-15]	9 (6) [3-15]	0.910
E2 (ng/L)	45 (15) [15-88]	49 (29) [15-88]	0.559
Post-ET 12th-day beta hCG (U/L)	193.2 (229.8) [27.9-880.1]	51.7 (91.6) [9.3-398.6]	**0.001**
Post ET 14th-day beta hCG (U/L)	567.8 (533.3) [64.0-2213.5]	141.2 (231.5) [0.9-1694.0]	**0.001**
Beta hCG ratio	2.67 (0.86) [1.63-9.77]	2.05 (2.23) [0.07-5.62]	**0.003**
IVF indication, n (%)			
Unexplained	35 (43.2)	16 (37.2)	0.695
Poor ovarian reserve	18 (22.2)	13 (30.2)	
Male factor	24 (29.6)	13 (30.2)	
Tubal factor	4 (5.0)	1 (2.3)	
Pregnancy outcome, n (%)			
Miscarriage	11 (13.6)		
Live birth	70 (86.4)		

BMI: body mass index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, ET: embryo transfer, hCG: human chorionic gonadotropin, FHB: fetal heartbeat, IVF: in vitro fertilization, descriptive statistics for continuous variables were presented as median (interquartile range, 75th-25th percentile) [minimum-maximum]

Table 4. Comparison of pregnancies resulting in birth and abortive pregnancies

	Birth (n=70)	Abortion (n=54)	p
Age (year)	29 (8) [21-42]	30 (9) [20-43]	0.298
BMI (kg/m^2)	23.5 (2) [20-28]	24 (3) [21-29]	**0.035**
Duration of infertility (year)	5 (3) [1-23]	6 (6) [1-16]	**0.026**
FSH (U/L)	7 (3) [4-15]	7 (5) [4-16]	0.471
LH (U/L)	9 (4) [4-15]	9 (4.25) [3-15]	0.286
E2 (ng/L)	45 (17.25) [24-88]	48.5 (21) [15-86]	0.328
Post-ET 12th-day beta hCG (U/L)	210.0 (208.5) [36.4-880.1]	55.5 (111.6) [9.3-398.6]	**<0.001**
Post ET 14th-day beta hCG (U/L)	586.3 (520.6) [101.0-2213.5]	146.4 (282.6) [0.9-1694.0]	**<0.001**
Beta hCG ratio	2.65 (0.90) [1.63-9.77]	2.29 (1.88) [0.07-5.62]	**0.005**

BMI: body mass index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, ET: embryo transfer, hCG: human chorionic gonadotropin, descriptive statistics for continuous variables were presented as median (interquartile range, 75th-25th percentile) [minimum-maximum]

Table 5. Evaluation of the performance of beta hCG values in predicting biochemical pregnancy, fetal heartbeat, and live birth outcomes after embryo transfer

Result/Measurement	Cut-off	Sensitivity	Specificity	AUC	95% CI	p
Biochemical Pregnancy						
Post-ET 12th-day beta hCG	43	70.3	93.8	0.901	0.840 - 0.962	**0.001**
Post ET 14th-day beta hCG	175	92.5	86.6	0.952	0.910 - 0.993	**0.001**
Beta hCG ratio	2.06	77.7	85.5	0.855	0.750 - 0.959	**0.001**
Fetal Heartbeat						
Post-ET 12th-day beta hCG	58.6	87.7	55.8	0.818	0.741 - 0.895	**0.001**
Post ET 14th-day beta hCG	214.5	82.7	74.4	0.837	0.761 - 0.914	**0.001**
Beta hCG ratio	2.19	80.2	53.5	0.661	0.546 - 0.776	**0.003**
Live Birth						
Post-ET 12th-day beta hCG	145.5	77.7	70.0	0.808	0.732 - 0.885	**0.001**
Post ET 14th-day beta hCG	216	70.3	87.1	0.828	0.753 - 0.904	**0.001**
Beta hCG ratio	1.91	42.5	91.4	0.646	0.542 - 0.749	**0.005**

ET: embryo transfer, hCG: human chorionic gonadotropin, AUC: area under the curve, CI: confidence interval

level on post-ET day 12 also showed significant predictive value, but the highest AUC value was obtained from the day 14 measurement. Prediction of live birth outcomes revealed that both post-ET day 12 and day 14 beta hCG levels, and also beta hCG ratio had significant prognostic value. In particular, the post-ET day 14 beta hCG level with a cut-off value of 216 U/L was labeled as a critical predictor of live birth, showing a sensitivity of 70.3%, a specificity of 87.1%, and an AUC of 0.828 (95% CI, 0.753-0.904, p=0.001). The highest performance to predict all three results was at the post-ET beta hCG level that was measured on day 14 (Figure 1).

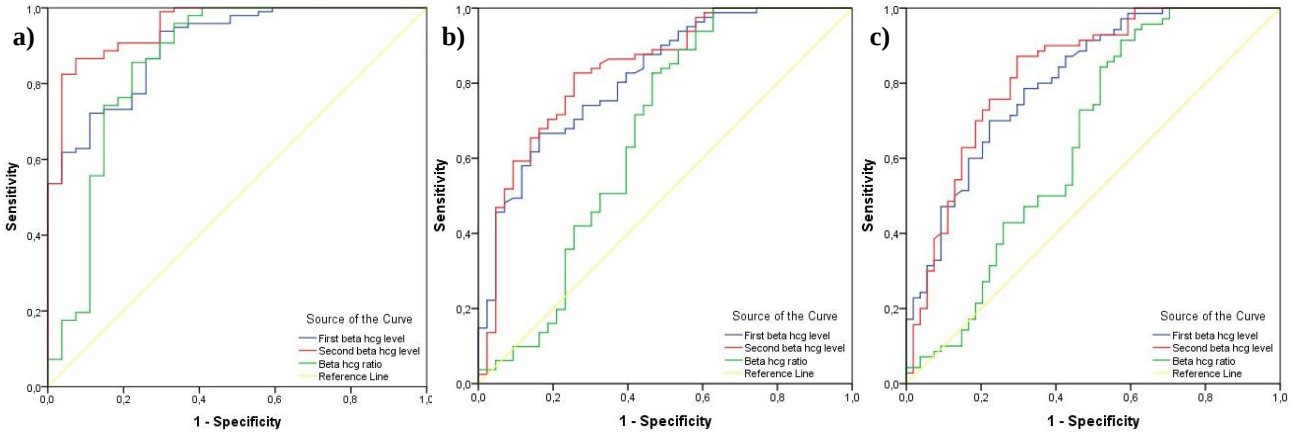

Figure 1. Receiver operating characteristic curve of first beta hCG, second beta hCG, and second to first beta hCG ratio to predict **a)** biochemical pregnancy, **b)** fetal heartbeat, and **c)** live birth

DISCUSSION

HCG is secreted by trophoblasts during the blastocyst stage, and its primary function is to keep the corpus luteum intact until the placenta matures enough to take over progesterone production. HCG is a heterodimeric glycoprotein composed of alpha and beta subunits derived primarily from syncytiotrophoblast cells. The beta subunit determines hCG's biological specificity (8). Years ago, it was known that a beta hCG of more than 100 mlU on the estimated menstrual date predicted a viable pregnancy (9). Patients, particularly in IVF pregnancies, are frequently required to take serum beta hCG tests to monitor the progress of conception, which increases the number of visits and thus causes psychological and financial stress. To reduce patient anxiety and costs, follow-ups must be tailored to individual serum beta hCG values. We aimed to present the ideal beta hCG cut-off values in IVF cycles that resulted in pregnancy, as well as any early pregnancy complications that may occur so that clinicians could guide their patients.

In this study, we discovered that beta hCG levels measured on the 12 and 14 days after day 3 single ET, as well as fold changes, were significantly higher in the live birth patient group than the biochemical pregnancy group. There are useful studies in the literature that attempt to predict the course of pregnancy by taking single or multiple beta hCG measurements during IVF cycles (10-14).

In this study, we discovered that the first (post-ET day 12) beta hCG value was 58.6 U/L, and the second (post-ET day 14) beta hCG value was 214.5 U/L, which predicted the FHB. Poikkeus et al. (15) found that a beta hCG level of 76 mIU/mL predicted a viable pregnancy with a sensitivity of 80% and a specificity of 82% when measured 12 days after ET. This value is slightly higher than found in this study. Bjercke et al. (16) found 55 IU/l, Qasim et al. (17) found 42 mlU/ml, and Sugantha et al. (18) found 50 IU/l, which are consistent with the findings in this study for a viable pregnancy.

Another retrospective study by Urbancsek et al. (19) found that the beta hCG value after IVF treatment was 50 IU/L for ongoing pregnancies and 135 IU/L for multiple pregnancies. In a retrospective study investigating the relationship between pregnancy and serum beta hCG value on the post-ET 12th-day, the mean serum beta hCG value was reported as 126 IU/L in single viable pregnant women, while 31 IU/L in nonviable pregnant women (19). In this study, we discovered a beta hCG level of 43 IU/L, with a sensitivity of 70.3% and 93.8% specificity to differentiate a nonviable pregnancy on the post-ET 12th-day.

Sung et al. (20) discovered that the cut-off value for postovulatory 12th-day beta hCG levels to predict live birth was 40.5 mIU/mL, with 75.2% sensitivity and 72.6% specificity. In addition, they discovered that a postovulatory 14th-day beta hCG level of 104.5 mIU/mL predicted a live birth with 80.3% sensitivity and 74.1% specificity. Hughes et al. (21) discovered that when beta hCG levels "doubled" in 48 hours, a live birth occurred in 80.7% of IVF cycles, and when beta hCG levels "reached 100" 15 days after oocyte retrieval, a live birth occurred in 81.6% of IVF cycles. In this study, post-ET 14th-day beta hCG was the most accurate predictor of live birth, with an optimal cut-off value of 216 IU/L. Similar to the present study, Grin et al. (22) found that the best value for predicting live birth for beta hCG measured on the 14 and 16 days after fresh ET is 211 IU/L (sensitivity 84%, specificity 76.2%) and 440 IU/L (sensitivity 86.0%, specificity 72.5%), respectively.

The second to first beta hCG ratio had the lowest prediction rate, with a cut-off value of 1.91 for live births. Sung et al. (20) discovered that the beta hCG changes in biochemical pregnancy, early pregnancy loss, and live birth groups were 2.0±1.3, 3.0±1.0, and 3.1±0.8 fold, respectively. Our study revealed that the fold changes for biochemical pregnancy, clinical pregnancy, and live birth were 2.06, 2.19, and 1.91, respectively. In our study, the beta hCG folding rate for live birth was lower than that of Sung et al. (20). They also discovered that the post-ET 14th-day sensitivity of serum beta hCG levels to predict ongoing pregnancy was 72.2% and 73.6%, respectively. When the initial beta hCG value was taken as 347 mIU/ml, we discovered that the post-ET 14th-day beta hCG cut-off value for live birth was 216 IU/L.

Kathiresan et al. (23) discovered that the proposed optimal thresholds predictive for live birth were 94 IU/L post-ET 12th-day; the likelihood of live birth on day 3 ET with beta hCG levels >94 IU/L was 79%. The beta hCG level in this study, which was measured on the post-ET 12th-day and

can predict live birth, was 145.5 IU/L, with a sensitivity of 77.7% and a specificity of 70%.
The limitation of this study was the fact that studied with a small number of patients. The strength of the study was the exclusion of multiple ETs and pregnancies, which could cause a false beta hCG increase.

CONCLUSION

It is critical to be able to predict the correct probabilities of pregnancy in IVF patients while reducing their anxiety. The earliest predictor of pregnancy outcomes in IVF cycles is early beta hCG measurement, which represents trophoblastic mass and function. This study yielded reliable data that can assist clinicians with beta hCG results and beta hCG ratio on the 12^{th} and 14^{th} days following ET. Because live birth is the desired outcome in IVF pregnancies, clinical and laboratory parameters that predict live birth are critical. The post-ET 14^{th}-day beta hCG level was discovered as the best predictor of live birth with a higher sensitivity.

Ethics Committee Approval: The study was approved by the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences (08.04.2022, 22/209).

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Metabolic Differentiation in Manic Episode of Bipolar Disorder Compared to Substance-Induced Psychosis and Substance Use Disorder Based on Serum Valproate Level

Bipolar Bozukluğun Manik Epizodunda, Maddeye Bağlı Psikoz ve Madde Kullanım Bozukluğu ile Karşılaştırıldığında Serum Valproat Düzeyine Göre Metabolik Farklılaşma

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ABSTRACT

Aim: Valproic acid (VPA) is primarily used in the treatment of epilepsy but also has uses in the treatment of manic episodes in bipolar disorder and substance use disorders. Manic episodes and psychosis may also affect hepatic clearance and drug distribution volume. The aim of this study was to assess the effect of mania and psychosis compared to substance use on VPA pharmacokinetics, specifically changes in total and unbound clearance.
Material and Methods: Fifty patients with a manic episode of bipolar disorder, and 51 patients with substance use disorder, 38 of whom were considered as substance-induced psychosis, were included in this retrospective study. All patients received a constant dose of 1000 mg VPA daily for at least five days, and serum VPA concentrations were measured.
Results: The mean serum levels of VPA were 59.2±17.4 µg/ml in the substance use disorder group, 60.9±13.5 µg/ml in the substance-induced psychosis group, and 61.8±13.7 µg/ml in the manic episode of bipolar disorder group. No significant difference was found between the groups (p=0.840). When considering substance use disorder and substance-induced psychosis as one group, the mean VPA level of 60.5±14.4 µg/ml in this group showed no significant difference compared to 61.8±13.7 µg/ml in the manic episode of bipolar disorder (p=0.630).
Conclusion: After reaching steady-state plasma levels, no significant difference in serum VPA levels was observed between the three groups. This suggests that manic episodes do not lead to a significant increase in VPA metabolism compared to substance use disorder or substance-induced psychosis.
Keywords: Serum valproate level; manic episode of bipolar disorder; substance use disorder; substance-induced psychosis.

ÖZ

Amaç: Valproik asit (VPA) öncelikle epilepsi tedavisinde kullanılır, ancak aynı zamanda bipolar bozukluktaki manik atakların ve madde kullanım bozukluklarının tedavisinde de kullanımları vardır. Manik ataklar ve psikoz hepatik klerensi ve ilaç dağıtım hacmini de etkileyebilir. Bu çalışmanın amacı, madde kullanımına kıyasla mani ve psikozun VPA farmakokinetiği, özellikle toplam ve bağlanmamış klerensteki değişiklikler üzerindeki etkisini değerlendirmektir.
Gereç ve Yöntemler: Bu geriye dönük çalışmaya bipolar bozukluk manik atağı olan 50 hasta ve 38'i maddeye bağlı psikoz olarak değerlendirilen madde kullanım bozukluğu olan 51 hasta dahil edildi. Tüm hastalara en az beş gün boyunca sabit dozda günlük 1000 mg VPA verildi ve serum VPA konsantrasyonları ölçüldü.
Bulgular: Ortalama serum VPA düzeyi madde kullanım bozukluğu grubunda 59,2±17,4 µg/ml, maddeye bağlı psikoz grubunda 60,9±13,5 µg/ml ve bipolar bozukluğun manik döneminde ise 61,8±13,7 µg/ml idi. Gruplar arasında anlamlı bir fark bulunamadı (p=0,840). Madde kullanım bozukluğu ve maddeye bağlı psikoz tek grup olarak ele alındığında bu grupta ortalama 60,5±14,4 µg/ml olan VPA düzeyi, bipolar bozukluğun manik dönemindeki 61,8±13,7 µg/ml ile karşılaştırıldığında anlamlı bir fark göstermedi (p=0,630).
Sonuç: Kararlı durum plazma seviyelerine ulaştıktan sonra, üç grup arasında serum VPA seviyelerinde anlamlı bir fark gözlenmedi. Bu, mani epizodunun madde kullanım bozukluğu veya maddeye bağlı psikoz ile karşılaştırıldığında VPA metabolizmasında anlamlı bir artışa yol açmadığını düşündürmektedir.
Anahtar kelimeler: Serum valproat düzeyi; bipolar bozukluğun manik dönemi; madde kullanım bozukluğu; maddeye bağlı psikoz.

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INTRODUCTION

Valproic acid (VPA) is a short-chain branched fatty acid used in the treatment of psychiatric disorders, migraine, neuropathic pain, and seizures (1). It is effective in the treatment of acute manic episodes of bipolar disorder and used in the long-term prevention of relapse (2). Valproate has also been used for the improvement of abstinence and impulse control in substance use disorder (SUD) (3). When combined with psychosocial therapies, it can be a safe and effective treatment for cocaine dependence (4).

The pharmacokinetic profile of VPA is complex: VPA has a low clearance (CL) due to high albumin binding (5) and the serum concentration of physiologically active free VPA changes in a non-linear manner (6). At least three different processes are involved in the almost complete first-order kinetic metabolism of valproate in humans, including glucuronidation, mitochondrial oxidation, and oxidation mediated by cytochrome P450s (7). It increases brain GABA levels (8,9), which can induce a sedative state and reduce anxiety and may be useful in the treatment of moderate mania (10,11). Valproate also inhibits voltage-gated sodium ion channels (12), blocks calcium channels, and modulates serotonergic and dopaminergic neurotransmission (8,9). Valproate may inhibit the activity of an enzyme (active protein kinase) that has been associated with an increase in cell surface area in people with bipolar disorder (13-16). Valproate, a fatty acid, has also been suggested to influence lipid metabolism in the brain (17). Beyond short-term biochemical effects, there appears to be consistent and strong evidence that valproate works through long-term effects at the genomic level (18,19).

There are no approved indications for its use in addiction psychiatry. However, its use in SUDs is increasing (20). Valproate is prescribed by consultant psychiatrists to reduce abstinence scores and to help with impulse control problems in patients hospitalized for substance-induced psychosis (SIP) and SUDs. Studies have shown that manic episodes in bipolar disorder affect drug pharmacokinetics. This can lead to increased CL of drugs such as lithium and carbamazepine. Clinical effects of VPA and serum drug concentrations are closely correlated, with significant inter-individual variability influenced by variables such as age, total body weight, and concomitant medication. Patients who do not respond to treatment are given higher doses/serum levels than patients who have responded to lower doses/serum levels (channeling effect) (21). Studies of lithium (22) and carbamazepine (23) show that serum levels of these drugs are lower, and CL is higher in acute mania. Similarly, the pharmacokinetics of carbamazepine have been shown to be affected in acute mania, with a significantly higher CL/bioavailability ratio than in epileptic patients (24).

Research on the effects of VPA in different conditions is limited. This is the first study to assess the effects of mania and psychosis on the pharmacokinetics of total and unbound (u) VPA, which may predict changes in total CL and unbound clearance (CLu). We hypothesized that serum levels of VPA will be lower in patients with manic episodes of bipolar disorder and SIP than in patients with SUDs, as we expect VPA CL to increase during mania or psychosis. If we find differences in serum valproate levels in different disorders, serum VPA levels can be used as a diagnostic marker and the initial dose of VPA can be adjusted according to the different disorders. Therefore, this study aimed to assess the effect of mania and psychosis compared to substance use on VPA pharmacokinetics specifically changes in total and CLu.

MATERIAL and METHODS

Participants were recruited from Erenköy Mental and Nervous Diseases Training and Research Hospital during a therapeutic drug monitoring male psychosis service. The following inclusion criteria were met by all patients seen at the male psychosis service: 1) receiving 1000 mg of VPA daily for at least five days, 2) being between 18 and 65 years of age, 3) weighing between 50 and 80 kg, 4) being male patients, and 5) smoking. The exclusion criteria were as follows: 1) previous valproate treatment, 2) patients with abnormal renal function tests (serum creatinine >1.2 mg/dl in adult males and 1.1 in adult females), 3) patients with abnormal liver function tests (AST and ALT >2.5 times of normal), 4) use of drugs that interfere with VPA metabolism (eg. phenytoin, phenobarbital, felbamate, ethosuximide, acyclovir and rifampine), 5) severe medical history, and 6) patients with a history of bipolar mood disorder and those with substance use problems.

Two psychiatrists diagnosed the patients with manic episodes of bipolar disorder, SUD, and SIP based on the Diagnostic and Statistical Manual of Mental Disorder, Fifth Edition (DSM-V) criteria during the hospitalization period. The DSM-V defines a SIP as a psychiatric disorder with delusions and/or hallucinations during or within one month after the intoxication or withdrawal of a substance, and that the substance can produce the symptoms. Patients with SIP whose psychotic symptoms persisted after one month were included in our study. We included patients receiving valproate for the first time in their lives. Sodium valproate was prescribed to each study participant on the recommendation of their psychiatrist and according to standard practice.

We retrospectively searched the data of the Erenköy Mental and Nervous Diseases Training and Research Hospital for four years until we selected a total of 101 patients according to the study criteria; 51 patients with substance use problems, of whom 38 with SIP and 13 with SUD, and 50 patients with manic episode of bipolar disorder. A psychiatrist verified the diagnoses by means of a review of each patient's records. This helped to ensure that the diagnoses were correct. As described above, the study also had strict exclusion criteria. Almost all patients received injections of haloperidol and biperiden as needed during the first few days of treatment. The study was approved by the ethics committee of Erenköy Mental and Nervous Diseases Training and Research Hospital (dated Nov 3, 2014, approval number: 18/8).

Blood Sampling and Drug Assays

The patients' laboratory tests were performed on the first morning of treatment. For the measurement of serum VPA concentration, serum samples were taken before administration of the morning dose and 12 hours after administration of the evening dose on days 5 to 7 of valproate treatment at 1000 mg/day. Sodium valproate concentration was determined after samples were thawed to room temperature. The sodium valproate concentration

was determined after thawing the samples to room temperature. The photometric methods were carried out using the Abbott Architect ci4100. VPA serum levels below or above 50 μg/ml (lowest effective dose) were also categorized.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess whether a variable is normally distributed. Normally distributed variables were reported as mean±standard deviation and non-normally distributed variables were reported as median (interquartile range) [minimum-maximum]. To compare two groups of normally distributed quantitative variables an independent samples t-test was used. For a parametric comparison of the three groups for quantitative variable ANOVA with Tukey's post hoc test was used while Kruskal-Wallis was used for the non-parametric comparison of the three groups. The chi-squared test was used to determine whether there is a significant association between two or more categorical variables. Fisher's exact test was used when more than 20% of the cells had an expected number of less than 5. Spearman correlation was used for the association between nonparametric measures of rank correlation. IBM SPSS v.21.0 was used to calculate all statistics. The significance level was set at $p<0.05$.

RESULTS

Fifty hospitalized patients with manic episodes of bipolar disorder and a total of 51 hospitalized patients with SUD and SIP were recruited for the study. Among the 51 patients with substance use, 38 patients had psychotic features and were diagnosed with SIP, while the other 13 were diagnosed with SUD.

In the comparison of patients with SUD, patients with SIP, and patients with manic episodes of bipolar disorder with each other in three different groups, the median age was significantly different between the three groups (p=0.003), while there was no significant difference between the three groups in terms of disease duration (p=0.370). When the education status was categorized as having studied for less than or more than 8 years, there was no significant difference between the three groups when comparing the groups (p=0.370). Also, there was no significant difference in terms of employment status when considered as working or not working (p=0.503) and in terms of marital status (p=0.156) between the three groups (Table 1).

Regarding the three groups in terms of pharmacological treatment received in addition to VPA treatment, almost all patients were receiving antipsychotics. In the SUD group, 2 patients were receiving risperidone, 4 patients were receiving olanzapine, 2 patients were receiving other antipsychotics, 3 patients were receiving combined antipsychotic treatment, and 2 patients were not receiving any medication other than VPA. In the SIP group, 6 patients received risperidone, 12 patients received olanzapine, 17 patients received combined antipsychotics and 3 patients received antidepressants. In the manic episodes of bipolar disorder group, 11 patients received risperidone, 7 patients received olanzapine, 6 patients received other antipsychotics, 23 patients received combined antipsychotics, 1 patient received antidepressants, and 2 patients were treated with VPA alone (Table 2). There is no difference between one type of medication and a combined type of medication between the three groups (p=0.175).

Table 1. Demographic characteristics of the groups

	BMP (n=50)	SUD (n=13)	SIP (n=38)	p
Age (years)	32 (16.5) [19-59]	25 (5) [20-36]	25 (7.25) [15-43]	**0.003**
Duration of illness (years)	2 (12.5) [0.5-24]	4 (6) [1-25]	2.5 (5) [0.5-10]	0.370
Education, n (%)				
≤8 years	21 (42.0)	8 (61.5)	20 (52.6)	0.370
>8 years	29 (58.0)	5 (38.5)	18 (47.4)	
Working status, n (%)				
Working (or student)	32 (64.0)	6 (46.2)	23 (60.5)	0.503
Nonworking (or retired)	18 (36.0)	7 (53.8)	15 (39.5)	
Marital status, n (%)				
Single (or divorced)	34 (68.0)	11 (84.6)	32 (84.2)	0.156
Married	16 (32.0)	2 (15.4)	6 (15.8)	

BMP: bipolar manic phase, SUD: substance use disorder, SIP: substance-induced psychosis

Table 2. Medication of three different groups besides VPA

	BMP (n=50)	SUD (n=13)	SIP (n=38)	p
Medication, n (%)				
One type	26 (52.0)	10 (76.9)	18 (47.4)	0.175
Combined type	24 (48.0)	3 (23.1)	20 (52.6)	
One type of medication, n (%)				
Risperidone	11 (22.0)	2 (15.4)	6 (15.8)	
Olanzapine	7 (14.0)	4 (30.8)	12 (31.6)	
Other Antipsychotics'	6 (12.0)	2 (15.4)	0 (0.0)	
No other medication	2 (4.0)	2 (15.4)	0 (0.0)	
Combined type of medication, n (%)				
Combined Antipsychotics	23 (46.0)	3 (23.1)	17 (44.7)	
Antidepressants+Antipsychotics	1 (2.0)	0 (0.0)	3 (7.9)	

VPA: valproic acid, BMP: bipolar manic phase, SUD: substance use disorder, SIP: substance-induced psychosis

The substances used in the last three months by those with SUD and those with SIP were cannabinoids, synthetic cannabinoids, volatile substances, psychostimulants, and a combination of these (Table 3). The difference between the two groups in terms of single or mixed substance use was not significant (p=0.734).

The mean serum levels of VPA were 59.2±17.4 μg/ml in the SUD group, 60.9±13.5 μg/ml in the SIP group, and 61.8±13.7 μg/ml in the manic episode of bipolar disorder group (Table 4). No significant difference was found between the groups for serum valproate levels (p=0.840). When VPA levels were categorized as less than or greater than 50 μg/ml, no significant difference was found between the three groups (p=0.310).

No significant correlation was found between age and VPA concentration (r_s=-0.092, p=0.361). Also, there was no significant correlation between disease duration and VPA concentration (r_s=-0.031, p=0.757).

When considering SUDs and SIPs together as one group of substance use (Table 5), the median age was found statistically significantly different between the SUD+SIP and manic episode of bipolar disorder groups (p=0.001), while there was no significant difference in median disease duration between these two groups (p=0.780). The mean VPA level was found 60.5±14.4 μg/ml in the SUD+SIP group showing no significant difference compared to 61.8±13.7 μg/ml in the manic episode of bipolar disorder group (p=0.630). Also, no significant difference was found between two groups when VPA levels were categorized as less than or greater than 50 μg/ml (p=0.667).

Table 3. Substances used in the last three months in SUD and SIP groups

	SUD (n=13)	SIP (n=38)	p
Substance, n (%)			
One type	4 (30.8)	10 (26.3)	0.734
Mixed type	9 (69.2)	28 (73.7)	

SUD: substance use disorder, SIP: substance-induced psychosis

DISCUSSION

In this study, the difference in VPA pharmacokinetics and pharmacodynamics between groups may provide some clues to the mechanism of manic episodes of bipolar disorder, SUD, and SIP. Although we observed that serum VPA levels may vary in the clinical setting, we did not find a significant difference in serum VPA levels between the manic episode of bipolar disorder patients compared with SUD and SIP patients, and we did not find a relationship between drug pharmacokinetics.

They can affect it in two different ways. First, increased release of several neurotransmitters, including norepinephrine and other catecholamines, may increase hepatic blood flow during the acute episode of mania. The dose rate divided by the trough total VPA concentration was used to calculate the CL. The CLu, or intrinsic CL, was calculated as the ratio of the dose to the unbound concentration of VPA. VPA is a hepatically metabolized drug. It has a low extraction ratio. CLu is the intrinsic or metabolic CL. VPA levels are similar in all three groups, indicating that changes in liver perfusion caused by drug and other medication use do not affect sodium valproate serum levels. Other variables affecting hepatic metabolism are medication and age. Higher rates of antipsychotic medications and substance usage might have influenced the entire results by affecting hepatic metabolism. All three groups' antipsychotic medications were like each other which is compatible with our results that it did not change the serum level of VPA. Even though the median age of the groups was like each other, all in young adulthood, it was statistically different among groups of SUD and SIP, and manic episodes of bipolar disorder. We could not find a relationship between the serum concentration of VPA and age, and duration of illness. According to reports, there is a nonlinear relationship between VPA dosage and serum concentration, which means that serum concentration did not rise proportionately as the dose increased. The patient's blood drug concentration may not rise as expected when the drug dose is increased; this could be because the drug CL rate has also increased (25).

Table 4. Comparison of VPA concentration in three different groups

	BMP (n=50)	SUD (n=13)	SIP (n=38)	p
VPA concentration (μg/ml)	61.8±13.7	59.2±17.4	60.9±13.5	0.840
VPA concentration, n (%)				
<50 μg/ml	10 (20.0)	5 (38.5)	7 (18.4)	0.310
≥50 μg/ml	40 (80.0)	8 (61.5)	31 (81.6)	

VPA: valproic acid, BMP: bipolar manic phase, SUD: substance use disorder, SIP: substance-induced psychosis

Table 5. Age, duration of illness, and VPA Concentration in two different groups

	BMP (n=50)	SUD+SIP (n=51)	p
Age (years)	32 (16.5) [19-59]	25 (6) [15-43]	**0.001**
Duration of illness (years)	2 (12.5) [0.5-24]	4 (6) [0.5-25]	0.780
VPA concentration (μg/ml)	61.8±13.7	60.5±14.4	0.630
VPA concentration, n (%)			
<50 μg/ml	10 (20.0)	12 (23.5)	0.667
≥50 μg/ml	40 (80.0)	39 (76.5)	

VPA: valproic acid, BMP: bipolar manic phase, SUD: substance use disorder, SIP: substance-induced psychosis, descriptive statistics were reported with mean±standard deviation for normally distributed variables, and median (interquartile range, Q_3-Q_1) [minimum-maximum] for nonnormally distributed

In bipolar acute manic episodes, abnormalities in membrane transport systems and secondary messenger systems reduce erythrocyte Na+/K+/ATPase activity (23), increasing the volume of drug distribution and decreasing serum valproate levels. VPA is often used as a first-line treatment in patients with rapid cycles. As VPA has a limited therapeutic window, therapeutic drug monitoring is an essential component of drug therapy. It appears to be effective in treating mania at serum levels between 50 and 125 μg/ml, but the risk of toxicity increases above 125 μg/ml (26). It appears that the pharmacokinetics of some drugs may change depending on the bipolar disorder state (27).

Studies support the use of VPA in the treatment of cocaine dependence and alcohol dependence. It has been shown to be successful in preventing relapse. It has also been effective in controlling impulsivity and irritability, making it useful in the treatment of people with borderline personality disorder who are more likely to develop alcohol or drug use disorders. Patients with cocaine dependence may have lower levels of GABA. Several mechanisms in VPA favor the synthesis of GABA, increasing its release and the postsynaptic GABAergic response (20). All these findings support the use of valproate in SUDs or SIP.

Drug-disease interaction is a revolutionary strategy that has just come into vogue. Acute manic-bipolar illness affects the pharmacokinetics and pharmacodynamics of some drugs (28). Estimation of volume of distribution for one-compartment models and peripheral volume of distribution for two-compartment models. Although absorption of VPA is almost complete. Body weight, VPA dose, and age were significant covariates reported to influence the volume of distribution. The volume of distribution of VPA increased with increasing body weight and VPA dose. Saturable protein binding could explain the increased volume of distribution at higher doses (29). Consistent with our results, another study found that neither trough sodium valproate concentration nor internal CL changed between individuals in the acute manic episode and those in the maintenance period. On the other hand, patients with manic episodes require higher doses of VPA to achieve serum concentrations compared with patients with epilepsy (23).

Whether the patients' medication can be considered an important confounding factor. There was no significant difference between the groups in the medications used for the three disorders. Valproate monotherapy is as effective as antipsychotics and lithium in acute mania, but the combination of valproate and an antipsychotic is more effective than either drug alone. Valproate monotherapy has comparable efficacy to olanzapine in the maintenance treatment of bipolar disorder, although placebo-controlled evidence is limited. If an acute episode responds to the combination, maintenance treatment with valproate and quetiapine or olanzapine is more effective than valproate alone. Valproate may reduce plasma SGA concentrations (30). The addition of aripiprazole to lithium or valproate had no clinically meaningful effect on the pharmacokinetics of either drug (31). Smoking is very common in psychiatric inpatients. The prevalence of tobacco use is higher (36.1% versus 21.4%) in people with mental illness than in healthy controls (32). Although smoking does not affect the pharmacokinetics of VPA, we only included smoking patients in order to reduce one of the confounding factors affecting drug levels with other drug interactions. Smoking may induce CYP1A2 enzymes, thereby reducing the expected plasma levels of certain second-generation antipsychotics. Valproate is a minor substrate of CYP2A6, 2B6, 2C9, 2C19, and 2E1 and is almost completely metabolized with first-order kinetics (33). Smoking may have a direct effect on drugs metabolized by CYP1A2, such as olanzapine. It is unclear exactly how valproate and cannabinoids interact with one another. Valproate and cannabinoid treatment together had no discernible impact on each other's plasma levels or metabolites (34). There is no literature regarding the interaction between VPA and the other substances used.

In the study by Machino et al. (35), bipolar I and II patients were successfully treated with stable doses of valproate as prophylactic therapy for at least 12 months. Valproate levels may approximate appropriate valproate levels, and there may be a relationship between the amount of valproate required for stabilization and the subtype of bipolar disorder. In maintenance treatment, the bipolar I disorder group showed a greater trend towards a trough serum valproate level than the bipolar II disorder group.

Valproate was more effective than placebo in preventing the new episodes of mania or depression in bipolar disorder but did not differ significantly from lithium, second-generation antipsychotics, or other anticonvulsants. Overall, the benefits in bipolar depression were not significantly greater than those in mania (36). For the first time, a case series questions the depressogenic potential of valproate in people who have recovered from a severe manic episode. If a patient experiences depressive symptoms after recovery from a manic episode, a reduction in valproate dose should be considered as a therapeutic approach. The cases also show that lower valproate doses and serum levels are effective in the maintenance phase of bipolar disorder compared with the acute manic episode. It is emphasized that different valproate doses and serum levels may be therapeutic in different stages of bipolar disorder (37).

Although we did not find a significant difference, a recent study by Hsueh et al. (38) demonstrated the role of the dopaminergic system in bipolar disorder and the effect of VPA on this system at both clinical and preclinical levels. In euthymic bipolar disorder patients treated with VPA, there was a negative correlation between VPA concentration and striatal dopamine transporter (DAT) availability, whereas bipolar disorder patients had an increased level of striatal DAT availability compared to controls. These findings suggest that the DAT system, specifically DAT availability, is important in the pathophysiology of bipolar disorder. It is involved in VPA-mediated physiological changes. Therefore, a common mechanism in the pathophysiology of bipolar disorder and the therapeutic mechanism of VPA may be mediated by DAT availability. DAT homeostasis may represent a novel therapeutic strategy for bipolar disorder patients.

Ho et al. (39) conducted a genome-wide association study to understand the effects of antiepileptic drugs on mood stabilization in bipolar disorder patients and suggested a possible influence on drug absorption, suggesting that antiepileptic drugs may alter drug pharmacokinetics.

The limitation of this study is that it was conducted only with male patients that sex differences could not be evaluated. VPA level was examined only in the manic period of bipolar disorder. Even though all the patients are in young adulthood, and all are smokers, the age difference was significantly different between groups, and the amount of cigarette smoking is not evaluated in the study. Patients were treated with higher rates of antipsychotic medications by suggested guidelines and algorithms; however, antipsychotic medications may have affected the entire pattern of results. Additional latent but unmeasured variables may have contributed to the current pattern of results like body weight. Finally, if the study had been carried out over a longer period, and with a larger sample of patients, a difference might have been noticed.

CONCLUSION

The main findings of this study are that the concentration of sodium valproate in patients with manic episodes of bipolar disorder, SUD, and SIP did not differ significantly between groups. Mania or psychosis does not lead to a significant increase in VPA metabolism compared to substance use. As we cannot exclude the effects of substance use on liver metabolism, future studies may include patients with impulse control disorder or epilepsy in the comparison group.

Ethics Committee Approval: The study was approved by the Ethics Committee of Erenköy Mental and Nervous Diseases Training and Research Hospital (03.11.2014, 18/8).

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A Case of Hantavirus Renal Syndrome Detected in the COVID-19 Pandemic

COVID-19 Pandemisinde Saptanan Hantavirüs Renal Sendrom Olgusu

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ABSTRACT

Hantaviruses are enveloped RNA viruses in the Bunyaviridae family that cause rodent-borne zoonotic infections. They cause two separate diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS), as a result of transmission to humans through contact with rodent excrements and inhalation. The form seen more common in Türkiye is HFRS, which progresses with acute kidney injury and thrombocytopenia. Coronavirus disease 2019 (COVID-19) is an infectious disease that ranges from asymptomatic infection to pneumonia, respiratory failure, and death. Because of symptoms such as fever, weakness, and flu-like clinical findings in the early days, it can be confused with many infectious diseases. In this case report, a case of hantavirus renal syndrome admitted with fever, weakness, and flu-like symptoms during the COVID-19 pandemic was presented.
Keywords: COVID-19; hantavirus; thrombocytopenia.

ÖZ

Hantavirüsler Bunyaviridae ailesi içinde yer alan, kemirgen kaynaklı zoonotik enfeksiyonlara yol açan zarflı RNA virüsleridir. Kemirgen dışkıları ile temas ve solunum yoluyla insanlara bulaş sonucu renal sendrom ile seyreden kanamalı ateş (RSKA) ve hantavirüs kardiyopulmoner sendrom (HKPS) olmak üzere iki ayrı hastalığa yol açmaktadırlar. Türkiye'de daha çok görülen formu akut böbrek yetmezliği ve trombositopeni ile seyreden RSKA tablosudur. Koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) ise asemptomatik enfeksiyondan pnömoniye, solunum yetmezliğine ve ölüme kadar değişen klinik tablolara yol açan bir enfeksiyon hastalığıdır. Hastalık özellikle erken dönemde neden olduğu ateş, halsizlik, gribe benzer klinik bulgular gibi belirtiler nedeniyle birçok bulaşıcı hastalık ile karıştırılabilmektedir. Bu olgu sunumunda, COVID-19 pandemisi sırasında ateş, halsizlik ve grip benzeri klinik bulgular ile hastaneye başvuran bir hantavirüs renal sendrom olgusu sunulmuştur.
Anahtar kelimeler: COVID-19; hantavirüs; trombositopeni.

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INTRODUCTION

Viral hemorrhagic fevers are zoonotic infections that progress with fever and bleeding and are transmitted to humans mostly by ticks, mosquitoes, and rodents (1). Hantaviruses are enveloped RNA viruses that belong to the Bunyaviridae family and cause viral hemorrhagic fever. It is transmitted to humans mainly by contact with

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body exudates and secretions of infected rodents, and by inhalation (2). In addition, more rarely, it can be transmitted as a result of a rodent bite. Except for hantavirus pulmonary syndrome (HPS), which is caused by the Andes subtype, it is not transmitted from person to person by contact (3). While hantaviruses cause asymptomatic chronic infection in rodents, they occur in humans with two clinical manifestations characterized by fever with renal hemorrhage and pulmonary syndrome (4). However, nonspecific symptoms such as fever, and malaise in the prodrome stage of the disease before the present picture develops create a clinical similarity with the early stages of many diseases. In this report, a case that was initially followed up as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and then diagnosed with hemorrhagic fever with renal syndrome (HFRS) during the coronavirus disease 2019 (COVID-19) pandemic was presented.

CASE REPORT

A 57-year-old male patient with no known disease was admitted to an external clinical center with fever and flu symptoms five days ago. A COVID-19 polymerase chain reaction (PCR) sample was taken and symptomatic treatment was prescribed. He was admitted to the emergency room of our hospital with complaints of fever, fatigue, and abdominal pain. At the time of admission, body temperature was 38.5 ºC, heart rate was 118/min, arterial blood pressure was 120/75 mmHg, respiratory rate was 18/min, and other system examinations were normal. In laboratory tests, white blood cell (WBC) 11500/uL, neutrophil 7500/uL, platelet (PLT) 53000/uL, C-reactive protein (CRP) 11 mg/dL (0-0.5), urea 112 mg/dL, creatine 3.5 mg/dL, aminotransferase (ALT) 11 IU/L, aspartate aminotransferase (AST) 21 IU/L. The patient resides in the Yigilca district of Düzce, where there was a previous hantavirus epidemic, a history of going to the forest and drinking water in the forest 15 days ago. He was hospitalized with the preliminary diagnoses of hantavirus and leptospirosis. Hantavirus IgM, IgG, and leptospira PCR tests were requested. Methylprednisolone 80 mg 1x1 was started with the recommendation of internal medicine in the patient with urea 162 mg/dL, creatinine 9.1 mg/dL, and no urine output in the follow-ups, and the patient was taken to hemodialysis. Urea and creatinine values decreased after dialysis. After the results of leptospira PCR negative, hantavirus IgM (IFA) intermediate value and IgG (IFA) negative, the control hantavirus IgM and IgG antibodies studied one week later were both positive and hantavirus renal syndrome was diagnosed. During the follow-ups, the patient did not need dialysis, the patient in the polyuric phase was followed up with hydration and supportive treatment, the methylprednisolone dose was tapered off, and the patient was discharged on the 12th day of his hospitalization, whose symptoms and laboratory findings improved, and urea and creatinine values returned to normal.

DISCUSSION

The annual incidence of HFRS in the world is between 60 and 150 thousand and 90% of them are reported from China, Korea, and Russia. Almost 90% of the cases in Europe are reported from Scandinavian countries (Finland, Sweden, Norway). The presence of hantaviruses in wildlife rodents in Türkiye was first reported in a field study published in 2004. In a study conducted in Bartın, hantavirus seroprevalence was found to be 5.2%, and in a study conducted in Giresun, it was 3.2% (5).

While hantaviruses cause chronic asymptomatic infection in rodents, they cause two types of disease in humans. The incubation period of the HFRS form is 1-3 weeks, and there are five periods in the course of the disease: the febrile period, the hypotensive period, the oliguric period, the polyuric period, and the convalescent period. Fever, fatigue, and abdominal pain are the initial clinical findings. Although not all patients need dialysis, renal failure does not become chronic in patients who recover (6). Urea and creatinine elevation, thrombocytopenia, proteinuria, and hematuria are frequently detected in laboratory findings. In addition, high CRP values and leukocytosis, which are seen in many infectious diseases, can also accompany the disease (7). In our case, the disease started with prodromal complaints such as fever and malaise, followed by the need for hemodialysis as a result of increased urea and creatinine, followed by a polyuric period and recovery.

The incubation period in HCPS is between 2-3 weeks and there are four phases of the disease: the febrile/prodromal phase, the cardiopulmonary phase, the oliguric and diuretic phase, and the convalescent phase. The febrile period is characterized by fever, chills, and muscle pain. Increased pulmonary capillary permeability in the cardiopulmonary period leads to a decrease in cardiac output, acute respiratory distress syndrome (ARDS), and shock. This phase is also manifested by pulmonary edema, arrhythmias, and coagulopathy.

As with many viral infections, the diagnosis of hantavirus infection is made by serological tests and molecular tests. Demonstration of IgM-type antibodies in the serum in the acute phase of the disease or detection of at least a 4-fold increase in IgG titer in two separate serum samples taken during the acute and convalescence period is sufficient for the diagnosis of hantavirus infection. Another method is the detection of hantavirus RNA in serum and urine by reverse transcription-PCR (RT-PCR). The diagnosis of the presented case was made with positive results for hantavirus IgG and IgM (IFA) antibodies in the serum during the oliguric period.

The first human hantavirus epidemic confirmed clinically and serologically in Türkiye was detected in February 2009 in the Zonguldak-Bartin. 25 suspected cases were seen, 12 of them serologically detected Puumala virus (PUUV) subtype (7). Subsequently, two cases residing in Giresun, one of which was mortal, were reported, and the Dobrova virus (DOBV) subtype was found serologically in them (8). Finally, a hantavirus epidemic was observed in Düzce in April 2017, and the diagnosis of hantavirus was confirmed serologically in 20 of 50 suspected patients. While the PUUV subtype was detected in 18 of the patients, the subtype could not be determined in 2 patients. Of the 20 patients who were followed up with hantavirus positivity, 17 were living in the Yigilca district, which is defined as the hantavirus region, and the other three were living in neighboring districts. Except for one patient who died as a result of ARDS and cardiac arrest without renal involvement, the other patients were followed up with the diagnosis of HFRS and five patients (25%) received hemodialysis (9).

The present case is from the Yigilca district in April 2021 during the COVID-19 pandemic. The Yigilca is a region suitable for hantavirus infections due to its proximity to forests and habitats and the widespread spring water use. There are forest workers, farmers, and beekeepers in the risk groups for this disease. The presented case stated that he lived in a detached house in the countryside in the shade of the forest and drank the spring water from the forest.

Currently, there is no effective antiviral treatment for the hantavirus. However, in various studies, ribavirin treatment has been shown to reduce mortality (3). In hantavirus infections, treatment is limited to supportive treatment, and dialysis and platelet transfusion are applied when necessary. In this case, no antiviral treatment was applied for the hantavirus. Hydration and methylprednisolone therapy were used as supportive treatments. Hemodialysis was applied as a result of the patient's lack of urine output and increased creatinine. The patient, who entered the polyuric phase, did not need dialysis after the urea and creatinine values returned to normal.

Three hantavirus infections diagnosed in the COVID-19 pandemic have been reported in the literature (10-12). All cases are cardiopulmonary syndrome. All reported cases had a fever, thrombocytopenia, and bilateral pulmonary infiltration. And the history of hospitalization due to respiratory deterioration. While two cases were extruded after intubation and discharged, one case was fatal. The case reported from Argentina initially presented with headache and myalgia, and the SARS-CoV-2 PCR test was positive. In all of the cases, hantavirus tests were requested as a result of living in the endemic region, traveling to the endemic region, and learning about mouse contact in the detailed histories of the patients. While the diagnosis was made by serological methods in two cases, the diagnosis was confirmed by PCR in the mortal case. The present case was admitted with nonspecific symptoms such as fever, headache, and myalgia and was initially diagnosed with COVID-19. The patient had a negative SARS-CoV-2 PCR result and was tested for hantavirus at the first visit because he had thrombocytopenia and high creatinine and lived in the region where hantavirus is endemic. The patient with a positive hantavirus IgM, IgG test was diagnosed with hantavirus renal syndrome.

The present case is important in that it was first diagnosed as COVID-19 infection due to the pandemic period, and then diagnosed with a detailed history and clinical experience. Hantavirus infections should be kept in mind in the differential diagnosis of patients who live in or have a history of travel to the endemic region and present with fever, acute renal failure, and thrombocytopenia.

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Reviewing Delusional Misidentification Syndromes with Examples

Örneklerle Sanrısal Yanlış Tanımlama Sendromlarının Gözden Geçirilmesi

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ABSTRACT

Delusional misidentification syndrome is characterized by individuals perceiving familiar people, places, and objects as different entities, often associated with delusional disorders. These disorders are typically linked to abnormalities in cognitive processes, resulting in incongruent and unalterable beliefs. The loss of familiarity is believed to be the consequence leading to the emergence of these disorders. While commonly associated with psychiatric illnesses, they are also frequently observed in conjunction with neurodegenerative diseases. Diagnosis is primarily established through clinical evaluation. However, cases of these syndromes pose a significant burden on caregivers. Therefore, it is crucial not to overlook the possibility of dementia in these syndromes. This consideration is vital for providing appropriate support and treatment to patients and their families. This case report aimed to provide a detailed examination of this topic by presenting five cases.
Keywords: delusional misidentification syndrome; delusional disorders; Capgras syndrome; mirror self-misidentification syndrome; Alzheimer's dementia; vascular dementia; Lewy body dementia.

ÖZ

Sanrısal yanlış tanımlama sendromu, bireylerin tanıdık insanları, yerleri ve nesneleri farklı varlıklar olarak algılamasıyla karakterize edilir ve sıklıkla sanrısal bozukluklarla ilişkilendirilir. Bu bozukluklar genellikle bireyin düşünce süreçlerindeki anormalliklerle ilişkilendirilir ve gerçekle uyumsuz ve değiştirilemez inanışlarla sonuçlanır. Bu bozuklukların aşinalığın kaybolması sonucu ortaya çıktığı düşünülmektedir. Genellikle psikiyatrik hastalıklarla ilişkilendirilseler de aynı zamanda nörodejeneratif hastalıklarla birlikte de sıkça görülür. Tanı, öncelikle klinik değerlendirme yoluyla konmaktadır. Ancak vakalar, bakım verenler üzerinde ciddi bir yük oluşturur. Dolayısıyla, bu sendromlarda demans olasılığının göz ardı edilmemesi önemlidir. Bu durum, hastalara ve ailelerine sağlanacak uygun destek ve tedavi açısından kritiktir. Bu olgu sunumunda beş olgu sunularak bu konuya dair detaylı bir değerlendirme sağlanması amaçlandı.
Anahtar kelimeler: Sanrısal yanlış tanımlama sendromu; sanrısal bozukluklar; Capgras sendromu; aynada kendini yanlış tanımlama sendromu; Alzheimer demans; vasküler demans; Lewy cisimcikli demans.

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INTRODUCTION

Delusional misidentification syndrome (DMS) is a delusional syndrome characterized by the mistaken identification or perception of familiar individuals, places, and objects (1,2). It can manifest in various forms: Capgras syndrome, which is characterized by the belief that a known person has been replaced by an impostor (3); Fregoli syndrome, where a stranger is mistakenly identified as a familiar person who has changed appearance (4); intermetamorphosis, where the belief is that two known

individuals have switched places (5); subjective doubles, where a person believes they have an identical twin who acts independently (6); and mirror self-misidentification syndrome, where one misidentifies their reflection as a separate person. Although these syndromes are primarily associated with psychiatric disorders, they have also been observed in neurodegenerative diseases and organic brain damage. DMS can accompany neurodegenerative diseases, particularly Lewy body dementia, vascular dementia, and Alzheimer's dementia. In order not to overlook dementia in these delusional syndromes that are more commonly associated with psychiatric illnesses, we aimed to present five cases to review this topic.

CASE 1

A 78-year-old right-handed male patient, who is literate, presented to the clinic with episodic memory complaints that have been occurring for three years, along with a behavioral impairment of greeting himself in the mirror as someone else. The problems started with complaints of losing objects three years ago, followed by difficulties in recognizing family members and rummaging through rooms. All his needs are taken care of by his relatives. His medical history includes known diabetes mellitus, epilepsy, stroke, coronary artery disease, and hypertension. There is no significant family history. Neurological examination is unremarkable. In the neuropsychological evaluation, the Beck depression inventory (BDI) score was 7 which indicated no clinical depression. Also, the mini-mental state examination (MMSE) score was 13 (moderate dementia). While spatial orientation is preserved, there are impairments in time orientation, registration memory, and perceptual organization. The clinical dementia rating (CDR) score is 16. A brain imaging was performed using a magnetic resonance imaging (MRI) was performed. Fazekas scale score, which is a scale that indicates the level of small vessel disease was stage 2, and global atrophy in MRI at the sequence of fluid-attenuated inversion recovery (FLAIR) was observed (Figure 1A). The coexistence of Alzheimer's dementia and vascular dementia was considered. Mirror self-misidentification syndrome was improved after the treatment by donepezil 10 mg/day and memantine 20 mg/day into the six weeks without any improvement in neurocognitive test scores.

CASE 2

A 79-year-old right-handed female patient, who is literate, presented to our clinic with complaints of hoarding items, walking naked at home and talking to someone else in the mirror, which has been occurring for two years but has worsened in the past eight months. During the inquiry, it was revealed that she had a pre-existing REM sleep behavior disorder, and within the past two years, she had developed misbehaviors such as rummaging through rooms, urinary incontinence, aimless wandering at home, and closing curtains due to the belief of being watched. Her self-care needs are taken care of by her relatives. She has a medical history of controlled hypertension. There is no significant family history. Neurological examination showed slowness in walking, mild bilateral rigidity, and echolalia. In the neuropsychological evaluation, the MMSE score was 9 (moderate-advanced dementia), and the BDI could not be performed due to inconsistency. The CDR score is 16. The Koedam score, which indicates atrophy of the posterior cerebral areas, was found to be stage 2 (Figure 1B). Lewy body dementia was considered in the patient. After rivastigmine 9.5 mg/day transdermal patch and 12.5 mg/day quetiapine treatment, misbehaviors including mirror self-misidentification syndrome were resolved in one month.

CASE 3

A 48-year-old right-handed male patient, who has received eight years of education, presented to the clinic with an increase in difficulties remembering proper names and a facial recognition impairment last six months. During the inquiry, it was revealed that he couldn't even recognize his mother's face, found her expressions strange, experienced difficulties with orientation, developed a lack of interest in his surroundings, and exhibited behavioral problems such as swearing in public. The symptoms were found to be gradual and progressive over two years. His functional abilities were preserved. He has a history of cerebrovascular event two years ago. There is a family history of an unspecified psychiatric illness in his sibling. Apart from neurocognitive disorders, right hemihypoesthesia was detected in the neurological examination. BDI score was found 28. Personal and actual information, as well as spatial and temporal orientation, were preserved. However, there were difficulties in verbal memory processes, including encoding and retrieval, while lexical fluency was relatively preserved but semantic fluency was severely impaired. Dorsal and ventral pathway functions related to visuospatial abilities were impaired. Cranial imaging was performed with an MRI diffusion sequence including FLAIR due to claustrophobia, revealing chronic encephalomalacic areas in the left parietal and right temporal region (Figure 1C-D). Vascular dementia was considered in the patient. Treatment was initiated with rivastigmine 4.6 mg/day transdermal patch and 50 mg/day sertralin. The rivastigmine dose was increased to 13.3 mg. The sertraline dose was not changed. Although facial recognition did not improve, swearing and apathy improved in three months.

CASE 4

An 86-year-old right-handed male patient, who has received 15 years of education, presented to the clinic with complaints of seeing and talking to things that are not present at home for six months. He believed that his relatives were replaced with other people for three months (Capgras syndrome). During the inquiry, it was revealed that he has been experiencing fluctuating orientation disorder, mixing up names, losing objects, difficulty in naming, confusion with directions, problems with financial calculations, and mistaking sheets for people for the past two years. He is able to take care of his self-care needs. He has a medical history of asthma and benign prostatic hyperplasia. There is no significant family history. Neurological examination showed limitations in upward gaze, festination, dizziness upon standing up from sitting, rigidity, and circumferential speech. In the neuropsychological evaluation, the BDI score was 5, and the MMSE score was 24. Personal and actual information and spatial orientation were preserved, but there were relative difficulties in time orientation, difficulties in retrieval in verbal memory processes, severe

impairments in semantic and lexical fluency, and impaired organization. The Koedam scale indicated stage 2-3 in brain imaging (Figure 1E). Although there was no change in the improvement in cognitive evaluation, Capgras syndrome significantly regressed three weeks after starting 4.6 mg/day transdermal rivastigmine treatment.

CASE 5

A 77-year-old right-handed male patient, who has received three years of education, presented to our clinic with the inability to recognize himself in the mirror and mistaking the reflection in the mirror for three months. During the inquiry, it was revealed that he has been experiencing repeated questioning and topic repetitions for at least five years. He has started to lose his sense of direction and is unable to leave the house alone, leading to a decline in his functionality. He requires assistance with self-care. There is no known medical condition. It was learned that his mother and sibling have complaints of unconfirmed forgetfulness. Neurological examination did not reveal any pathology. In the neuropsychological evaluation, the BDI score and MMSE score were 14. Personal and actual information, as well as spatial and temporal orientation, were moderately impaired. Simple attention and working memory processes were relatively preserved, but there were difficulties in encoding verbal memory processes. There was an impairment in the organization. The mesial temporal atrophy (MTA) score, which indicates hippocampal degeneration, was found to be stage 3-4 (Figure 1F). Alzheimer's dementia was considered in the patient. The donepezil/memantine combination was started with a dose of 5/10 and increased to a dose of 10/20 mg/day. There was no change in his misidentification.

DISCUSSION

Delusional misidentification syndrome is a delusional disorder in which individuals mistakenly identify familiar objects or people as something or someone else. It can be a neurological or psychiatric condition and is characterized by the delusional belief of misidentification. The common feature of all DMSs is the loss of familiarity. The most commonly seen form is Capgras syndrome, but other forms include Fregoli syndrome, intermetamorphosis, subjective doubles, and mirror self-misidentification syndrome. Among the cases we have encountered, case 4 was diagnosed with Capgras syndrome, and the other four exhibited the phenomenon of misidentifying oneself in the mirror.

In Capgras syndrome, individuals can not recognize a loved one or acquaintance and believe that they have been replaced by someone else. The phenomenon of misidentifying oneself in the mirror, on the other hand, is considered normal in animals and infants but is not expected in adults. It can be observed in conjunction with disturbances in self-perception and visual-spatial perception associated with dementia disorders (7). These mental disorders typically manifest as symptoms of neurological or psychiatric conditions. They have been described in schizophrenia and schizoaffective disorders, and although they are more commonly associated with psychiatric illnesses, they can also occur in Alzheimer's dementia, Lewy body dementia, vascular dementia, epilepsy, cerebrovascular events, and advanced Parkinson's disease. The prevalence of these disorders has been reported to range between 5% and 82%. A review of 260 case reports related to DMS found that 174 (66.9%) of the cases had Capgras syndrome. Among the cases, 73% had a diagnosis of schizophrenia, 26.4% had dementia, and 16.7% had mood disorders (8). In two other studies, the rates of these phenomena occurring in different neurodegenerative diseases were reported to be between 16.6% and 27.8% in Lewy body dementia, and 15.8% in Alzheimer's disease (9,10). In another series of 47 patients with Capgras syndrome, it was found that 81% of the patients had a neurodegenerative disease, with Lewy body disease being the most common, followed by Alzheimer's disease. In individuals without neurodegenerative diseases, DMS typically emerges at a younger age and has been associated with psychiatric disorders, cerebrovascular events, and illicit drug use (11). Among our patients presented, the primary neurological diagnosis was as follows; one had a combination of Alzheimer's disease and vascular dementia (Case 1), two had Lewy body dementia (Case 2 and Case 4), one had vascular dementia (Case 3), and one had Alzheimer's disease (Case 5).

The pathophysiology of DMS appears to involve a disconnection syndrome where the connection between emotional information processing and face recognition is disrupted. It has been found that patients particularly have difficulty recalling past experiences with a hypoactive limbic system in the right hemisphere (12). Neuroimaging studies have also shown abnormal face recognition in these patients (13). Based on these findings, it has been suggested that there is a disconnection (caused by neurodegenerative or functional lesions) between the occipitotemporal cortex (involved in face recognition) and limbic circuits (neural circuits involved in the control of emotional expression) (14). In Lewy body dementia, hallucinations and misperceptions are common and are associated with Lewy body pathology in specific anatomical areas, which can involve disrupted cortical connections between the occipital and temporal lobes (15). A study examining 17 patients with Capgras syndrome based on antemortem imaging also found a neuroanatomical disconnection between impaired familiarity processing (left retrosplenial cortex) and belief evaluation (right frontal cortex) (16).

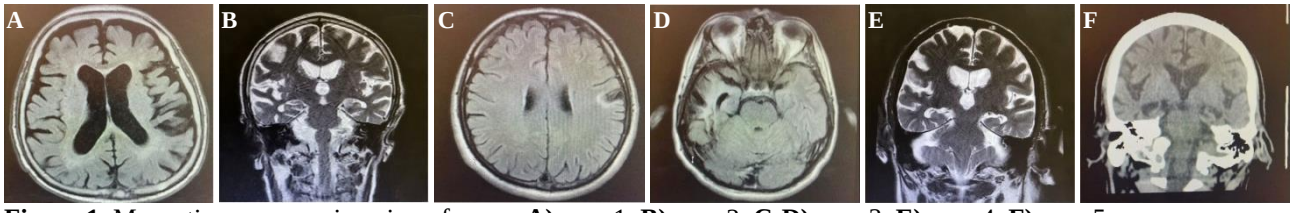

Figure 1. Magnetic resonance imaging of cases, **A)** case 1, **B)** case 2, **C-D)** case 3, **E)** case 4, **F)** case 5

DMS is a clinical diagnosis, and its diagnosis is based on the clinical evaluation of symptoms (8). DMS is one of the most challenging clinical findings for caregivers. In our five patients, the complaints related to these delusional syndromes were the reason for seeking medical attention, despite the presence of other symptoms before. This condition, which disrupts the trust relationship between the caregiver and the patient more than the loss of recognition itself, undermines the effectiveness and safety of care and intensifies the burden on the caregiver (17). The patient does not allow assistance with self-care from someone they perceive as an imposter or fake. Confusion, fear, and anger lead to the rejection of attention from the caregiver, and in some cases, it can even result in aggressive behaviors (17,18). Alternatively, individuals with mirror self-misidentification syndrome may feel anger towards their family members who try to convince them that they are using their own belongings at their own homes. Although there was no aggression in our patients, they exhibited persecutory feelings towards their caregivers.

Pharmacological or non-pharmacological interventions have been shown to not completely eliminate DMS. However, antipsychotic medication combined with therapies that make the patient feel safe, such as music or reminiscence therapy, as well as transcranial magnetic stimulation methods, have been tried (19,20). Approaching the patient with empathy and showing interest in their anxieties related to their condition can enhance the effectiveness of treatments (21). Hospitalization is also an option in cases where the patient poses a risk to themselves or others. We have observed benefits from symptomatic treatments of dementia including acetylcholine esterase inhibitors and memantine in DMSs in dementia cases. Due to the overlap of their current symptoms with psychiatric disorders, our patients remained untreated in the neurology department for a long time, highlighting the importance of recognizing that these conditions can also be seen in neurodegenerative diseases.

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Neuraxial Block in A Post-Hemorrhagic Stroke Pregnant Patient

Hemorajik İnme Sonrası Gebe Bir Hastada Nöroaksiyel Blok

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ABSTRACT

The selection of anesthetic methods for labor and delivery in individuals with elevated intracranial pressure relies on careful consideration of the risks and benefits. While neuraxial analgesia and anesthesia are favored for healthy individuals, they might not be suitable for individuals with intracranial lesions or a heightened risk of bleeding. Neuraxial block in post-stroke patients raises a concerning question about its safety due to the risk of herniation. The risk of perioperative major vascular events and mortality between general anesthesia and regional anesthesia in post-stroke patients is comparable. However, the neuraxial block shows benefits regarding airway manipulation and lower risk of thromboembolism. A successful and safe neuraxial anesthesia in a pregnant patient with a history of hemorrhagic stroke was presented in this case report.
Keywords: Post-stroke; neuraxial anesthesia; pregnancy; neuroanesthesia.

ÖZ

Kafa içi basıncı yüksek olan bireylerde doğum sancısı ve doğum için anestezi yöntemlerinin seçimi, risklerin ve faydaların dikkatli bir şekilde değerlendirilmesine dayanır. Nöroaksiyel analjezi ve anestezi sağlıklı bireyler için tercih edilirken intrakranial lezyonları olan veya kanama riski yüksek olan kişiler için uygun olmayabilir. İnme sonrası hastalarda nöroaksiyel blok, fıtıklaşma riski nedeniyle güvenliği konusunda endişe verici bir soru ortaya çıkarmaktadır. İnme sonrası hastalarda genel anestezi ile bölgesel anestezi arasında perioperatif majör vasküler olay ve mortalite riski karşılaştırılabilir. Bununla birlikte, nöroaksiyel blok, hava yolu manipülasyonu ve daha düşük tromboembolizm riski açısından faydalar göstermektedir. Bu vaka raporunda hemorajik inme öyküsü olan gebe bir hastada başarılı ve güvenli nöroaksiyel anestezi sunulmuştur.
Anahtar kelimeler: İnme sonrası; nöroaksiyel anestezi; gebelik; nöroanestezi.

INTRODUCTION

When considering anesthesia management in a post-stroke patient several factors need to be considered to ensure patient safety and optimize outcomes, especially in a special case such as pregnant patients. This equilibrium can be upset by pathologic changes in brain tissue, cerebrospinal fluid, or cerebral blood volume, which may lead to significantly elevated intracranial pressure (ICP), brain tissue shifts, or rupture of intracranial vascular lesions (1). Neuraxial block can cause brain herniation in patients with increased ICP which leads to worse outcomes. This case report aimed to present a successful and safe neuraxial anesthesia in a pregnant patient with a history of hemorrhagic stroke.

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CASE REPORT

Twenty-five years old female came into labor in the 33rd week of her second pregnancy with twins. She was admitted two weeks prior with an intracranial hemorrhage volume of 16 ml in the right parietooccipital lobe due to arteriovenous malformation rupture and discharged seven days later (Figure 1). Patient symptoms included moderate headache, vomiting, with no loss of consciousness, neurological deficits, and seizure was reported.

No sequelae symptoms were reported by the patient and the patient was not on any medication. The patient was able to do daily physical activity normally. The patient's weight was 70 kg and height was 165 cm with a BMI of 25.7 kg/m^2. The rest of the physical examination and laboratory examination were unremarkable. The computed tomography (CT) scan was not done due to patient refusal due to fetal radiation exposure, and magnetic resonance imaging (MRI) was not able to be done because the patient was already in labor.

The patient's optic nerve sheath diameter (ONSD) was measured in the morning before surgery and the result was within normal limits (Figure 2). The recent MRI was initially planned to be done, but the patient was then premedicated with paracetamol IV 1000 mg, ondansetron 4 mg, and midazolam 2 mg IV. Neuraxial anesthesia with 10 mg of bupivacaine hyperbaric 0.5% was done at L 2-3 level using a 29G spinal needle. A pinprick test was done and showed analgesia at the T8 dermatome level. No hypotension after spinal was observed and hemodynamic was stable during one hour of surgery.

No neurological symptoms were reported by the patient postoperatively. The patient was admitted to the ICU for postoperative monitoring for 24 hours before discharge to the ward (Figure 3). During the thirty-day follow-up, the patient had no complaints of neurological symptoms and was able to do her normal activity.

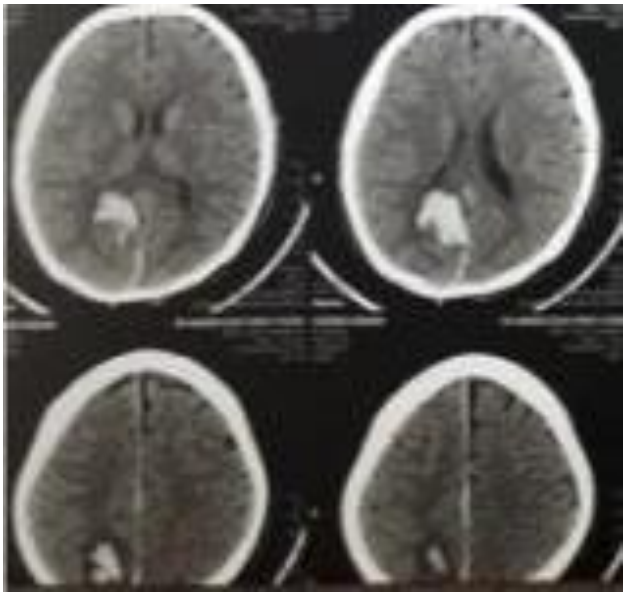

Figure 1. Computed tomography scan of two weeks before

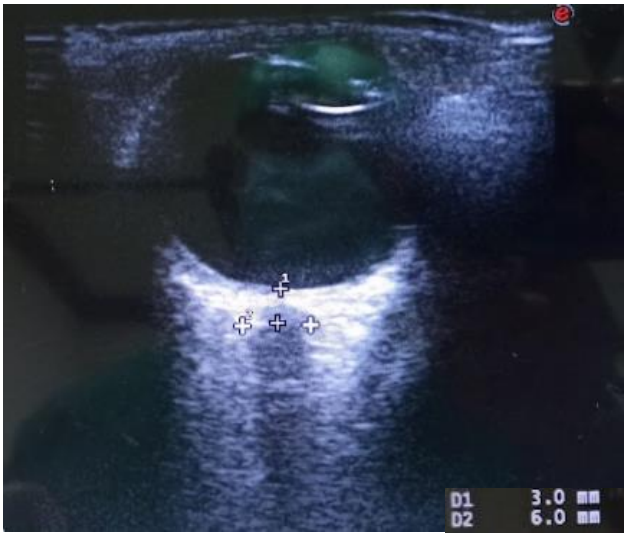

Figure 2. Optic nerve sheath diameter of 6 mm

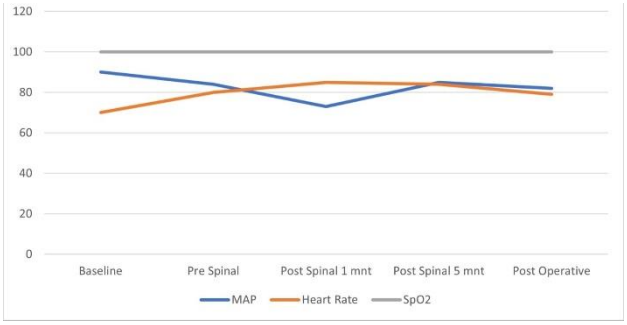

Figure 3. Postoperative monitoring in the intensive care unit

DISCUSSION

Timing of the Surgery and Anesthesia

In general, it is recommended to wait for a minimum of three to six months after an acute stroke before performing surgery and anesthesia, due to the impairment of autoregulation. The perioperative major vascular event and mortality rate are higher in patients within nine months post-stroke than in non-stroke patients. The highest perioperative major vascular event is higher within days 3-14 post-stroke (2). In this patient, the onset was right at 14 days and the patient was in an emergency situation because she was in labor and manual vaginal delivery can increase ICP during delivery, which outdone the risk of the surgery (3).

Anesthesia Consideration

Neuraxial block in patients with a history of neurological diseases was dilemmatic. There's a high risk of brain herniation in patients with high ICP. However, several studies have shown the benefits of neuraxial anesthesia compared to general anesthesia, especially in patients with a history of cerebrovascular diseases, such as minimal pulmonary complication and lower risk of DVT and pulmonary embolism. The dysphagia and diminished cough reflex in stroke patients and the muscle paralysis and sedation from general anesthesia (2,4,5). After throughout neurological examination, the patient showed no symptoms of increased ICP and we measured the ONSD before the spinal to help us exclude the high ICP. ONSD is a fast and non-invasive method for detecting elevated ICP. Several studies and meta-analyses have shown

consistent results that ONSD is a reliable examination. In one of the studies done by Kerscher et al. (4), it was found that the sensitivity of ONSD in detecting increased ICP was higher than funduscopy (92% vs 46%), but funduscopy is more specific for high ICP (86.4% vs 100%) in comparison to the ONSD.

The recent imaging, if possible, is preferably done to confirm the patient's current state. The patient's stroke was two weeks from the onset and showed full recovery, which lowers the risk of herniation. Ten mg dose of spinal is enough to reach T8 level block due to abdominal compression to the epidural space and a high level of progesterone increases a pregnant patient's sensitivity to local anesthesia. To reduce the risk of multiple punctures and big punctures in the dura, spinal was done by the chief resident of anesthesia using a 29G needle. The surgery was also done by the chief resident of obstetrics and gynecology to minimize the duration of the cesarean section.

Neurological Examination

A comprehensive neurological assessment is necessary before considering neuraxial anesthesia in post-stroke patients. This assessment should include a detailed evaluation of motor and sensory function, coordination, cognition, and any other neurological deficits. The presence of significant residual deficits or ongoing neurological deterioration may influence the decision to proceed with neuraxial anesthesia (5,6).

In the case in which high ICP and neurological symptoms can be excluded with stable hemodynamics, neuraxial anesthesia is a safe choice in post-hemorrhagic stroke patients.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

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Pulmonary Alveolar Proteinosis Secondary to Chronic Ethylene Oxide Occupational Inhalation

Mesleki olarak Kronik Etilen Oksit İnhalasyonuna Sekonder Pulmoner Alveolar Proteinozis

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ABSTRACT

In this case report, a 40-year-old male patient with a persistent and productive cough lasting over 2 weeks, accompanied by dyspnea, who received an anti-tuberculosis drug regimen for 12 months without any clinical improvement at a different hospital before being referred to Persahabatan Central General Hospital was presented. In-depth clinical, and radiological investigations, the periodic acid-Schiff (PAS)-positive related to pulmonary alveolar proteinosis (PAP) confirmed through transbronchial biopsy (TBB). PAP is a rare lung disease with exceptionally low prevalence and incidence, Notably, the patient's occupational environment played a crucial role in the diagnosis, as we identified occupational PAP secondary to chronic inhalation of ethylene oxide in a poorly ventilated work setting and inadequate respiratory protection. The patient was administered inhaled filgrastim (1 vial) at four intervals over 30 days, yielding favorable and satisfactory clinical as well as radiological outcomes.
Keywords: Chronic ethylene oxide; pulmonary alveolar proteinosis; pulmonary lavage; transbronchial biopsy.

ÖZ

Bu olgu sunumunda, 2 haftadan uzun süredir devam eden inatçı ve prodüktif öksürüğün yanı sıra nefes darlığı şikayeti olan, Persahabatan Merkez Genel Hastanesi'ne sevk edilmeden önce farklı bir hastanede 12 ay boyunca anti-tüberküloz ilaç tedavisi alan ve herhangi bir klinik iyileşme göstermeyen 40 yaşında bir erkek hasta sunulmaktadır. Ayrıntılı klinik ve radyolojik incelemelerde, pulmoner alveoler proteinozis (PAP) ile ilişkili periyodik asit-Schiff (PAS) pozitifliği, transbronşiyal biyopsi (TBB) ile doğrulandı. PAP son derece düşük prevalansı ve insidansı olan nadir bir akciğer hastalığıdır. Yetersiz havalandırılan bir çalışma ortamında ve yetersiz solunum korumasıyla kronik etilen oksit inhalasyonuna ikincil olarak mesleki PAP belirlediğimiz hastada, özellikle hastanın mesleki ortamı tanıda çok önemli bir rol oynadı.
Hastaya 30 gün boyunca dört aralıkla inhale filgrastim (1 şişe) uygulandı ve olumlu ve tatmin edici klinik ve radyolojik sonuçlar elde edildi.
Anahtar kelimeler: Kronik etilen oksit; pulmoner alveoler proteinözis; pulmoner lavaj; transbronşiyal biyopsi.

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INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare and intriguing lung disorder where the delicate equilibrium of surfactant clearance and production is disrupted (1). Approximately 90% of PAP cases stem from the autoimmune variant, while secondary PAP accounts for 4%, congenital PAP for 1%, and the remaining 5% consists of undetermined PAP-like diseases (1). Autoimmune PAP is triggered by IgG anti-granulocyte macrophage colony stimulating factor (anti-GM-CSF) antibodies,

which lead to a decline in functional alveolar macrophages. On the other hand, secondary PAP lacks these antibodies but still experiences a reduction in effective alveolar macrophages (2). However, diagnosing PAP resulting from chronic ethylene oxide (ETD) occupational inhalation is exceedingly rare, making this case report particularly noteworthy. This case report aimed to present a PAP case secondary to ETD occupational inhalation.

CASE REPORT

A 40-year-old male with a history of 5 pack-years of tobacco use, presented with a productive cough for more than 2 weeks followed by shortness of breath (mMRC 3). He received an anti-tuberculosis drug regimen for 12 months without any clinical improvement at a previous hospital, before being referred to Persahabatan Central General Hospital. The patient has been screened for pulmonary tuberculosis yielding non-specific chest x-ray results with negative acid-fast bacilli (AFB) on sputum. On clinical examination, he was found to have clubbed fingers with oxygen saturation at rest breathing air of 80%, and late inspiratory crackles in the middle and lower fields of both lungs. A pulmonary function test was not conducted due to pandemic-related reasons. A repeated chest x-ray for evaluation and a contrast thorax computed tomography (CT) scan were obtained, showing an appearance of interstitial lung disease. Bronchoalveolar lavage (BAL) revealed the appearance of thoracic epithelial cells, leukocytes, and macrophages with a red blood cell background. Parenchymal lung tissue biopsy from transbronchial biopsy (TBB) revealed the appearance of alveolar space containing amorphous eosinophilic material (Figure 1). This histopathologic pattern was suggestive of PAP. Autoimmune marker tests including ANA, dsDNA, and RF showed negative results, and the IgG anti-GM-CSF test was not performed in Indonesia due to its unavailability.

The patient worked as a medical stem sterilizer with exposure to ETD for 6 months without adequate respiratory protection, thus leaving significant symptoms such as an irritative cough and conjunctival inflammation during and after work. There's no exposure to other agents. However, the patient didn't experience any difference in symptom severity whether he was at work or in a non-work environment. Based on comprehensive studies and his detailed work history, the diagnosis pointed towards secondary alveolar proteinosis attributed to chronic ETD exposure. Exposure avoidance has been carried out by the patient for 3 months but there was no clinical improvement. Due to the unavailability of GM-CSF in Indonesia, the patient underwent a treatment regimen consisting of four rounds of inhaled 1 vial filgrastim, a granulocyte colony stimulating factor (G-CSF), at 30-day intervals, resulting in favorable clinical and radiological outcomes (Figure 2). The treatment was well-tolerated and clinical improvement was achieved after 4 months. Cough and dyspnea decreased (mMRC 1), and oxygen saturation increased to 96%.

DISCUSSION

Pulmonary alveolar proteinosis (PAP) stands as a rare pulmonary disorder characterized by surfactant accumulation within the alveoli failure of clearance rather than increased production. This condition may manifest congenitally, secondary to other conditions, or linked to autoimmune factors. The clinical presentation of PAP can vary ranging from mild to severe and the symptoms are often not specific. The most common complaints include dyspnea and cough, reported in 39% and 21% of the patients respectively. Physical examination findings are often unremarkable, but cyanosis (25% to 30%), clubbing (30%), or inspiratory crackles (50%) may exhibit in some patients (1,2). The patient presented dyspnea, cough, clubbing, and inspiratory crackles.

Pulmonary function testing is not obligatory for the diagnosis of PAP, and it is not a specific indication for this condition. Chest radiography may reveal bilateral alveolar opacities in a perihilar and basilar distribution without an air-bronchogram (2). On CT, PAP often displays a fascinating pattern known as "crazy paving" featuring intralobular thickening and diffuse ground-glass opacities. When there

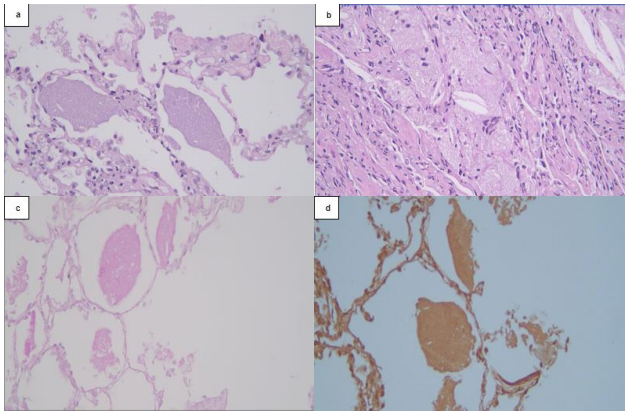

Figure 1. a,b) Haematoxylin & Eosin stain, the alveolar wall was relatively not thickened and the stroma was fibrotic and mildly infiltrated by leukocytes, **c)** periodic acid-Schiff stain, lumen filled with eosinophilic, **d)** Congo red stain was negative

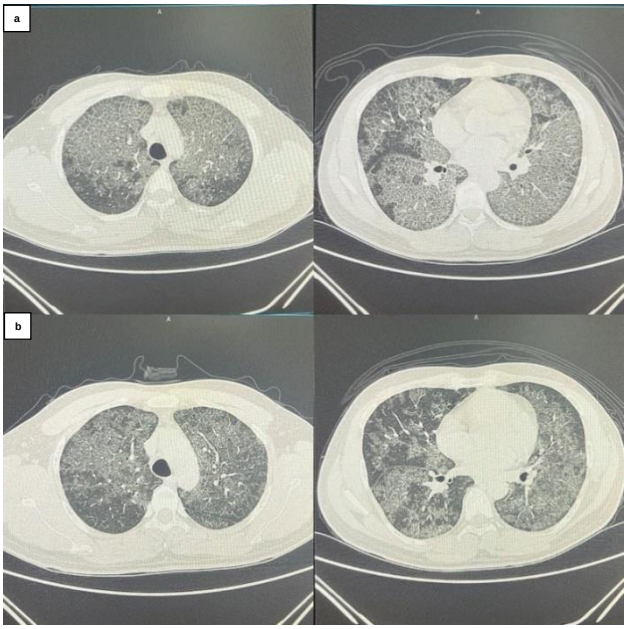

Figure 2. Axial view of thoracic computed tomography scan **a)** before and **b)** after granulocyte colony-stimulating factor (G-CSF) inhalation

is a suspicion of PAP, the gold standard for diagnosis is bronchoscopy with BAL. The lavage fluid often appears milky and opaque, and the cytological examination of BALF reveals large foamy macrophages with amorphous material that stains positive for periodic acid-Schiff (PAS) stain (2). A pulmonary function test was not conducted due to pandemic-related reasons. BAL revealed unspecific results, while TBB showed PAS-positive related to PAP.

Secondary PAP has been linked to various environmental exposures such as silica, talc, cement, kaolin, aluminum, titanium, indium, and cellulose. Studies from Japan and Korea have reported significant exposure rates in PAP, with 23% and 53% respectively (2,3). Raul et al. (4) and Li et al. (5) reported a case of PAP secondary to occupational inhaled exposure to chlorine and aluminum dust. ETD is an inhalation toxin that can induce various effects, including irritation of the eyes, skin, and mucous membranes (6). The patient's history of chronic ETD exposure at work left significant symptoms. There was no exposure to other agents. The patient didn't experience any difference in symptom severity whether he was at work or in a non-work environment. It suggests a potential association between exposure and the development of PAP. Additionally, exposure avoidance has been carried out by the patient for 3 months but there was no clinical improvement, and autoimmune marker tests showed negative results, although the IgG anti-GM-CSF test wasn't performed in Indonesia due to its unavailability.

Ndlovu et al. (7) reported a case of PAP diagnosis after re-evaluation for chronic cough unresponsive to empirical antituberculosis therapy. 4 of 7 patients were misdiagnosed with pulmonary tuberculosis before a diagnosis of PAP was made (8). As seen in previous cases, our patient was also treated with an anti-tuberculosis drug for 12 months without any clinical improvement at a different hospital before being referred to Persahabatan Central General Hospital. It could be misdiagnosed as tuberculosis because the symptoms of PAP can vary and are often not specific.

In exploring alternative therapies, clinical trials of GM-CSF replacement therapy have shown a positive response in 48% of a small group of 25 patients. Similarly, a trial involving 12 patients using inhaled GM-CSF revealed improvement in 91% of cases (2,9). In a fascinating study by Pamuk et al. (10), they explored G-CSF therapy in PAP patients with acute lymphoid leukemia. The use of G-CSF remarkably accelerated the patient's recovery leading to the disappearance of fever, a decrease in acute phase reactants, and the resolution of pulmonary infiltrates.

G-CSF is more commonly used than GM-CSF accounting for over >95% of the usage of molecularly cloned myeloid hematopoietic growth factors. This preference for G-CSF might be attributed to its wider usage and familiarity among physicians (11). In our study, we creatively employed inhaled filgrastim (G-CSF) by administering one vial four times over a 30-day interval due to drug limitations in Indonesia. The results were remarkable as the patient experienced significant clinical and radiological improvement demonstrating the effectiveness of this method. Further studies with larger sample sizes are necessary to make significant advancements in the future.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

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Author Contributions: Idea/Concept: FF, SA, WSH; Design: FF, RAP, ME, WSH; Data Collection/Processing: FF, ME, WSH, RB, MS; Analysis/Interpretation: FF, SA, PP, FFT, RB, MS; Literature Review: FF, RAP, SA, PP, FFT; Drafting/Writing: FF, RAP, RB, MS; Critical Review: RAP, ME, PP, FFT.

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SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
- Articles are required to have not been published in anywhere previously, and/or are not in the evaluation process for publication.
- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

ETHICAL RESPONSIBILITY

- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related with the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the article.
- Compliance of the articles with the scientific and ethical rules is responsibility of authors.

SUBMISSION FILES

Articles must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order in the article.

Cover Letter: Type of the article, the statement that has not been published previously in anywhere before, and/or not in the evaluation process for publication, if any, the people and institutions supporting the study financially and the relationship of these institutions with authors (if not, there is no relationship) must be stated. The names, academic titles, institutions, contact information and e-mail addresses of at least two reviewers suggested in relation to the subject of the article and not related to the authors and their institutions should be written. Editors' right to choose the reviewers are reserved.

Title Page: It must include the title of article (English and Turkish), short title not exceeding 40 characters, names, academic titles, ORCID® numbers, institutions, e-mail addresses of all authors, and also name, correspondence address, phone number, email address of the corresponding author. If the article has been presented previously in a scientific meeting; the name, date and place of the meeting (if not, not presented) should be stated.

Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables and Figures should be included.

Ethics Committee Approval Document: Ethics Committee Approval Document should be uploaded as a separate file for all research articles.

Note: If there are figures, pictures or photographs in the article, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including last table.
- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

DÜZCE
ÜNİVERSİTESİ

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