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Contents / İçindekiler

Review / Derleme

Research Articles / Araştırma Makaleleri

Case Report / Olgu Sunumu

REVIEW

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Reforms in Primary Health Care in Bulgaria - Past, Present, Future

ABSTRACT

The health of any nation, and the Bulgarian one in particular, is extremely important for the economy, security and development of the country. Unlike other European countries, due to defects in the structure and functioning, the Bulgarian health system could not achieve satisfactory final results in a number of important aspects of public health. One part of the essential problems includes high rates of morbidity, mortality, wide spread of health risk factors, and on the other - insufficient satisfaction of medical professionals (doctors, nurses, support staff) and patients. This, combined with the changes in the political administration of Bulgaria, led to a decision for a radical reform in the health care system. Conditionally, the changes can be divided, according to their nature, into 6 large groups: 1) Reforms leading to democratization of the system; 2) Reforms related to liberalization; 3) Reforms in the status of primary care practices; 4) Reforms in the organization, construction and structure of the system; 5) Reforms in financing and payment methods; 6) Reforms in the management of the primary care system and practices. The purpose of this review article is to present the theoretical framework, grounds and goals for the reform of the health care system in Bulgaria carried out in the past, with a focus on primary care and the current state.

Keywords: Primary Care, Health Care, Reforms in Primary Care.

Bulgaristan'da Birinci Basamak Sağlık Hizmetlerinde Reformlar - Geçmiş, Bugün, Gelecek

ÖZET

Herhangi bir milletin ve özellikle Bulgar milletinin sağlığı, ülkenin ekonomisi, güvenliği ve kalkınması için son derece önemlidir. Diğer Avrupa ülkelerinin aksine, yapı ve işleyişteki kusurlar nedeniyle, Bulgar sağlık sistemi, halk sağlığının bir dizi önemli yönünde tatmin edici nihai sonuçlara ulaşamadı. Temel sorunların bir kısmı yüksek hastalık ve ölüm oranlarını, sağlık risk faktörlerinin yaygın bir şekilde yayılmasını ve diğer tarafta tıp uzmanlarının (doktorlar, hemşireler, destek personeli) ve hastaların yetersiz memnuniyetini içerir. Bu, Bulgaristan'ın siyasi idaresindeki değişikliklerle birleştiğinde, sağlık sisteminde radikal bir reform kararı alınmasına yol açtı. Şartlı olarak, değişiklikler doğalarına göre 6 büyük gruba ayrılabilir: 1) Sistemin demokratikleşmesine yol açan reformlar; 2) Liberalleşmeye ilişkin reformlar; 3) Birinci basamak uygulamalarının durumundaki reformlar; 4) Sistemin organizasyonu, inşası ve yapısındaki reformlar; 5) Finansman ve ödeme yöntemlerinde reformlar; 6) Birinci basamak sisteminin yönetimi ve uygulamalarında reformlar. Bu gözden geçirme makalesinin amacı, birinci basamak ve mevcut duruma odaklanarak Bulgaristan'da geçmişte gerçekleştirilen sağlık sistemi reformunun teorik çerçevesini, temellerini ve hedeflerini sunmaktır.

Anahtar Kelimeler: Birinci Basamak, Sağlık Hizmeti, Birinci Basamakta Reformlar.

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INTRODUCTION

The health of any nation, and the Bulgarian one in particular, is extremely important for the economy, security and development of the country. Unlike other European countries, due to defects in the structure and functioning, the Bulgarian health system could not achieve satisfactory final results in a number of important aspects of public health. One part of the essential problems includes high rates of morbidity, mortality, wide spread of health risk factors, and on the other - insufficient satisfaction of medical professionals (doctors, nurses, support staff) and patients. This, combined with the changes in the political administration of Bulgaria after 1989 led to a decision to change the existing model of health care and carry out a reform at all levels - pre-hospital (primary and specialized) and hospital care. The good organization, financing and management of primary health care largely determines the functioning of the other floors of the system - secondary (specialized) and tertiary (hospital). Therefore, the beginning of the reform in the Bulgarian health care began with primary care in 2000, which followed a number of changes in the existing legislation of the country, as well as the appearance of new regulatory documents, structures and entities such as the national health insurance fund, practices in general medicine and general practitioners.

The purpose of this review article is to present the theoretical framework, grounds and goals for the reform of the health care system in Bulgaria carried out in the past, with a focus on primary care and the current state. The system is dynamic, which is why changes in it are not a single momentary act, but a sequence of actions. The analysis of the achieved results also shows the need to prepare a vision for its development in the future.

Theoretical Formulation, Foundations and Goals of the Reform: The structure, functioning and results of the activity of any health system are the product of the application of a certain system model (1).

The model is a theoretical "construction" that includes the main characteristics and properties that a health system should possess and reflects the main ideas and principles of a certain health doctrine. (2, 3, 4)

Over the last century, 3 main models have been used in the design of primary care systems:

- **Private-market model** - the healthcare system is a set of private medical institutions that work in market conditions and market competition. The producers of medical services (medical professionals) and the consumers (patients) are private individuals.
- **State-planned model** - the healthcare system is a collection of state-owned medical institutions, and its activity is organized on the basis of a state plan and state financing through taxes. There is no direct financial relationship between producers (medical professionals) and users (patients).
- **Social-market model (mixed model)** - the health system is a mixture of medical facilities with different ownership, state and private, in different ratios. Funding is mixed - health insurance, state and private. There are different financial relationships between producers (medical professionals) and consumers (patients). (2,4)

World experience shows that pure "private-market" and "state-planned" models lead to the creation of primary care systems that have many defects and negatively affect their efficiency and, consequently, individual and public health. (2)

In most European countries, primary care systems are built on the basis of the "social-market" model, with different ratios between state and private ownership, as well as between financing through health insurance, private and state payments. (5,6, 7- 9, 10)

Practice over the years has proven that this type provides opportunities for better efficiency and hence a greater contribution to people's health.

Health systems are complex and dynamic, constantly experiencing the impact of various external and internal factors (legal, economic, scientific, technological, socio-psychological, etc.) that change the conditions in which they work, and this forces them to constantly change to adapt to the new conditions.

These changes do not alter the main characteristics of the model on which the systems are built. They are a manifestation of good management, thereby creating better opportunities to achieve their goals - medical, economic, medical-social, psychological, social-psychological, etc. In the public space, very often they incorrectly present themselves for reform.

Reform is a type of ***change in the model of the health care system*** on which it is built.

As a technology, the reform is a ***set of different types and nature of activities*** (legal, economic, financial, organizational, etc.), ***through which the transformation of the systems is carried out.***

In Bulgaria, for more than 40 years, the primary care system has been built on the basis of the state-planning model (the so-called socialist model). The serious shortcomings of the model are the reason for the insufficient effectiveness of primary care in the recent past, which led to the deterioration of the health of the Bulgarian nation. (11,12)

In order to delay and stop this process, there was a public need for a reform of primary medical care. This became necessary with the political changes that occurred in Bulgaria after November 10, 1989 - "transition from totalitarianism and socialism to democracy and market economy".

The most important grounds for starting health care reform in Bulgaria are:

1) Change in the public and state system after 1989

2) Continuous deterioration of the indicators of the existing health

3) Unsatisfactory effectiveness of primary care

During the socialist period, large-scale health systems were created, with a high provision of certain types of resources (medical professionals), and with low economic capabilities of the state (insufficient funding).

Evidence analysis shows that the main reason for the lack of efficiency lies in the model that was used to build these systems. (13,14, 15,16, 4)

Gradually, the possibilities of primary care systems are exhausted because they do not contain the main driving forces for achieving higher quality and better efficiency:

• Existence of real economic interests of medical institutions, doctors and other medical professionals

• Existence of a market and market competition between medical institutions to stimulate their development and production of high quality services

• High economic and professional motivation of the medical staff

This is how it can be explained why the enormous efforts in recent decades did not lead to positive results in improving the activity of primary care medical facilities and their contribution to public health.

Primary care reform has a complex goal that can be decomposed into 4 consecutive sub-goals:

1. To be transformed according to the type of social market systems

2. To maintain good accessibility and increase the quality, effectiveness and efficiency of their activity

3. To increase its contribution to stop the processes of deterioration of public health

4. To improve the satisfaction of people, doctors and other medical professionals

The first ideas about the type of reforms appeared at the beginning of the democratic changes in Bulgaria after 1989.

In the political programs (1991-1997) of the main political parties, they proposed "solutions" for reforms - from minor to radical changes to the existing model and structure of the system, aligned with the doctrine of democratic and social market health care.

It was only in the period 1997-2000 that the shape of the reform was outlined and the main legal instruments for its implementation were created (the laws on health insurance and medical facilities, the Health Act, the Act on professional organizations of doctors and dentists, etc.). (17-21)

The reform can be considered, on the one hand, as a process of transformation of the model of primary care and on the other, as a set of changes of different types and nature - legal, organizational, managerial, economic, etc. with a view to implementing a selected model.

Content of the Reform in Primary Medical Care in Bulgaria: The beginning of the reform in primary medical care in Bulgaria was in 2000. Up to this point, the out-of-hospital system has been built on a regional basis. There were polyclinics that serve the population of a certain area of the settlement- city or village. They were staffed by doctors who have different specialties - internal medicine, pediatrics, cardiology and others. In the medical facility, there were nurses with the appropriate professional profile - pediatric, midwife and others. All medical specialists were employed on a contract at the polyclinic (health service), which is municipally owned, and had a fixed monthly remuneration (salary). Access to medical assistance was free and the patient himself decides and had the right to choose which specialist to turn to for consultation depending on the health problem.

This specialized model of medical care had a number of shortcomings, which is why a decision is made to carry out a reform, for which there was also the political will.

The content of the reform included various changes that led to the transformation of the existing system (state-planning) to the mixed social-market model.

Conditionally, the changes can be divided, according to their nature, into 6 large groups:

1) Reforms leading to democratization of the system;

2) Reforms related to liberalization;

3) Reforms in the status of primary care practices;

4) Reforms in the organization, construction and structure of the system

5) Reforms in financing and payment methods;

6) Reforms in the management of the primary care system and practices.

Each group included the mechanisms through which the primary care system is "transformed" with a view to achieving specific goals, namely greater efficiency and financial stability.

1. Reforms Leading to Democratization of Systems: They remove a number of existing restrictions and prohibitions on medical care left over from the totalitarian communist rule of the state, namely:

• The regional principle of receiving medical assistance according to place of residence and place of work

• The ban on private practice and private ownership of medical institutions

• The ban on free association by professional groups (doctors, nurses, dentists, etc.).

The following changes are the most important:

➢ ***The right of patients to freely choose a doctor and a medical facility,*** which lead to several important and essential consequences:

• The demand for medical assistance is determined by the patients according to their preferences, wishes, based on their own assessments (quality, living conditions, personal characteristics of the patients, etc.)

• Medical facilities become economically dependent on patients, which forces them to adapt to their preferences

• Conditions are created for the emergence of a market and market competition between medical institutions (doctors)

• significant changes in the behavior of medical institutions (primary care practices), placing the patient at the center of their activity

➢ ***The right of free professional association of doctors and other medical specialists***

Doctors are the main "producer" of medical services and are the most important for the good organization and management of the health system. This means that their active participation can only take place through free professional association.

In the socialist model, due to the fact that the state is a monopoly that "determines everything", doctors did not participate in an organized manner in making the most important decisions in health care.

Therefore, the restoration of the right to free professional association as a concrete manifestation of democratization is extremely important for changing the existing model. The restoration of this right takes place with the adoption of the Law on Professional Organizations of Doctors and Dentists, which leads to the restoration of the Bulgarian Medical Union. With it, the medical profession becomes an active participant both in carrying out the reform and in making decisions of a strategic nature.

After 2000 with the emergence of general practitioners, the need to create an association to protect the interests of this most numerous group of doctors in Bulgaria arises. This is how the National Association of General Practitioners in Bulgaria (NSOPLB) was registered, which expresses a professional expert opinion on issues related to general medical practice and actively defends its positions in front of the Ministry of Health.

2. Reforms Related to Liberalization: The liberalization of primary care involves an extremely diverse range of activities in the restructuring of ownership and of different types of activities.

Before the beginning of the reform in 2000, the state was the sole owner of the medical facilities and determined the construction, structure, resources in primary care. The state monopoly in ownership and management, combined with wrong management decisions, gradually leads to the exhaustion of the "potential" for providing quality and effective medical care. The state monopoly does not allow private money and ownership of medical institutions. Thus, health care is deprived of the driving forces of private interest, the market and competition, which has resulted in a deterioration of the quality and efficiency of outpatient care.

The removal of the state monopoly through the inclusion of new participants in outpatient care - the emergence of general practitioners, combined with changes in financing and payment models, are of fundamental importance in their transformation (remodeling). The right of free choice by patients of a general practitioner leads to the emergence of a market for outpatient services and market competition.

3. Reforms in the Status of Primary Care Practices: All medical facilities in primary care in 2000 become private - general practitioners found and register private medical facilities (primary care practice) under the Commercial Law and in this way they receive economic independence, autonomy in management, the right to profit, possibilities of bankruptcy and bankruptcy when the income does not cover the expenses, etc.

These changes lead to a total change of the existing model and from 2000 until now, primary medical care is based on private medical facilities that conclude a contract with the financing organization in Bulgaria - the National Health Insurance Fund.

The new commercial status of primary care practices requires radical changes in management, as the general practitioner is now the owner, and places him in a new position of manager. This necessitates the creation of a new attitude towards patients with a view to increasing their satisfaction and attracting new patients, etc., as well as the acquisition and improvement of new management skills, which are not typical for doctors up to this point.

4. Reforms in the Organization, Construction and Structure of Systems: The changes in the organization should lead to the transformation of the old unified medical system (primary, specialized and hospital care is simultaneously provided in one medical facility) into three independent systems with different objects of activity - the systems of primary, specialized out-of-hospital and in-hospital medical care.

The changes in the organization of the primary are of the most radical nature.

The model of the regional system (regional principle) has been replaced by the model of general medicine.

This shift has several ***important consequences:***

• Complete withdrawal of the state as a financing body and emergence of a health fund

• Emergence of a new type of doctor - general practitioner

• Creation of independent medical facilities-practices for primary medical care

• Introduction of free choice by patients of a general practitioner

• New sources of financing and methods of payment for the activities performed by the general practitioner

• Complete privatization of the activities performed by the general practitioner

• Partial privatization of property

The choice of this new model of primary care, based on the principles of general practice and carried out by a single doctor (general practitioner), has strong scientific and empirical grounds, as it has greater medical potential compared to the specialized model of the ward system and opportunity to apply the modern holistic approach.

During the implementation of the primary care reform, some mistakes were made, the consequences of which continue even now, 22 years after its beginning:

• No real advantages are created for disclosing group practices which led to a predominance of individual practices (about 95% of all practices in Bulgaria)

• A large package of medical and non-medical duties that can hardly be performed by one doctor, including and an obligation to provide 24-hour medical care under a contract with the Health Insurance Fund (22)

• Insufficient funds to finance primary care

• The reform started without giving the doctors who will work as general practitioners the opportunity to acquire the necessary competence in advance to work in general medical practice

• Difficult conditions for acquiring a specialty in general medicine (23)

• Introduction of rules that maximally limit the diagnostic and treatment freedom of general practitioners (24, 25)

The consequences of these mistakes continue to have a negative impact and create problems in primary care. Of greatest importance are the problems in the organization of medical assistance at night and on days off; limited access in some areas of the country (small villages in the mountains); the large number of referrals of patients to specialized medical care, the insufficient quality of clinical activities in some areas, etc.

5. Reforms in Financing and Payment Methods: Changes in financing and methods of payment of activities were essential to change the existing model of outpatient care.

The financial system was changing with the adoption of the Law on Health Insurance and introduction of the health insurance model.

The income and expenses for the financing of health care are separated from the tax and budget model and begin to be provided through insurance contributions (mandatory and voluntary), and the payment of medical facilities mainly through the budget of the National Health Insurance Fund or voluntary health insurance funds.

In this way, the state is separated from the direct financing (paying) of the medical facilities, and it remains a source of payment of insurance contributions only for certain categories of the population (children, pensioners, socially weak, etc.) and for payment of activities in certain types medical facilities (psychiatric, oncological, etc.).

The health fund became the main source of funding for primary care, from where it received more than 90% of its income. Radical changes in payment methods have completely replaced global budget financing.

Methods of payment for primary medical care in Bulgaria

The introduction of the model of general practice in primary care also required new payment methods that correspond to the nature and content of the activity in these medical facilities. (26,25)

When carrying out the health reform in Bulgaria, the experience of countries with multi-year systems based on general practice was used when choosing payment methods.

Primary medical care is financed through:

1) **Payment per Capita (per capita, capitation):** The capitation method of payment is established in the primary care systems, where the source of funding is the mandatory health insurance organizations (Bulgarian National Health Insurance Fund). It is a major prospective payment method that provides about 50% of revenue.

The payment of a capitation creates security for patients, providing them with the opportunity to use, if necessary, a certain package of medical care from the selected general practitioner, and the general practitioner thus undertakes to guarantee the performance of these activities. These are mainly activities related to the diagnosis and treatment of acute diseases. The method also creates a certain financial stability of the practices, since this is a guaranteed income that does not depend on whether the health-insured person will use the contracted services or not. The disadvantage is that the method is passive because it does not stimulate the activity of the general practitioner. There is net income for the practice if the health-insured person uses medical services less often.

The value of capitation payments is different according to age, and in our country people are divided into three age groups - from 0 to 18 years, from 18 to 65 years and over 65 years.

2) Payment for Medical Activities: Payment of certain medical activities performed in primary care is an active method. In contrast to capitation, when paying for activities, the more

patients and the more often they use this medical activity, the greater the income, respectively the profit for the practice. This stimulates the general practitioner to increase the amount of activities.

In Bulgaria, the method is applied by the Health Fund for payment of mandatory (since 2012 and recommended) immunizations, preventive examinations of children and adults, monitoring of normal pregnancy, monitoring examinations of patients with certain chronic diseases (arterial hypertension, ischemic disease of the heart, heart failure, type 2 diabetes, etc.).

World practice shows that, from an economic and medical point of view, the best ratio between capitation income and activity is 40:60. In Bulgaria, although it is gradually improving, this ratio is 55:45. (18,26)

3) Payment of User Fee (amount for each visit to the doctor): The introduction of this type of payment by the patient, or the so-called "copayment", has two functions:

- Regulatory - prevention of over-consumption, because although this financial "barrier" is low, it affects the person's decision to visit the doctor
- Co-financing - positive contribution to revenue

The user fee is paid by patients who have continuous health insurance rights for each visit to the general practitioner.

A large number of patients - children up to the age of 18, students, military personnel, socially weak, disabled, patients with various chronic diseases (experienced myocardial infarction with developed complications, experienced cerebral stroke with disability, etc.) are exempted from it, which is significant degree minimizes the expected effects of this type of payment.

At the start of the health reform in 2000 legal norms have been adopted that determine the amount of the user fee - 1% of the minimum wage for the country, and since 2012 a fixed amount independent of the minimum wage.

The user fee, as a type of co-payment by patients, is a continuous subject of public and political discussions regarding its increase, decrease or abolition.

And at the present moment, the budget of the Health Fund for primary care, as well as the prices for the activities performed by general practitioners, still do not correspond to the "priority" position of primary care in the health care system.

6. Reforms in Primary Care Management: The one-party political system before the reform (rule of the country by the Bulgarian Communist Party), combined with the state monopoly in health care (ownership, financing, etc.), determined the totalitarian nature of the management of the primary care system in Bulgaria. (19)

The specialized health authorities of central and local government (Ministry of Health and Municipalities), as owners, directly manage the entire system.

During communism in Bulgaria, the performance of management activities was linked to the achievement of a centrally imposed state plan. In this sense, "good governance" is that which brings about the implementation of the defined plan.

A major flaw in governance is the complete exclusion of doctors and other medical professionals and citizens from participation in decision-making.

The reforms in the country aim to increase the democratic and liberal character of the health systems, including primary medical care. The introduction of a new way of financing, the introduction of a commercial status of practices, the emergence of private medical institutions-practices in general medicine, the emergence of a market of medical services and competition, require the creation of a new type of management.

The goals of the management reform are threefold:

•Reduction of centralization - right to freely choose a general practitioner, regardless of place of residence

• Increasing democracy - possibility to make decisions about diagnosis and therapy at the level of general practice

• Creation of autonomy in management - the general practitioner is a doctor who is the manager of his practice

The main laws related to the implementation of the reform (the Law on Medical Institutions, the Law on Health Insurance, the Law on Professional Organizations of Doctors, etc.) become the legal basis for making changes in management.

Regardless of the goals set with the reform of primary care in Bulgaria, significant **current problems** still exist. The aging of general practitioners is significant, with the average age being around 60, and 88% being over 50. At the same time, the specialization in general medicine is associated with a number of defects, the training in general medicine in medical universities has insufficient hours provided in the programs, according to the uniform state requirements, 30 hours of lectures and 30 hours of exercises. This leads to low interest in this specialty and, accordingly, a small number of doctors enter the system. In addition, the "market" in large cities is distributed and it is difficult to start one's own practice, which is also associated with significant financial costs that are prohibitive for some young doctors, and this discourages them from this specialty. At the same time, there are regions of Bulgaria (especially the mountainous ones) where there are no people willing to work, and one general practitioner serves several villages, with

examinations only one day a week for a few hours. However, the state is not taking effective action to overcome these deficits, which are about to deepen. Problems also exist in the work organization of general practitioners. A large part of them share a common office, which necessitates the division of working hours, being in practice half a day. This greatly complicates the access of actively working patients. In group practices, there is no possibility to see another doctor, unlike in some countries, for example Great Britain. Due to increasing financial costs, a large number of doctors work without a hired nurse or other medical and non-medical staff, which makes it difficult and slows down the work, which also includes a number of non-medical activities, such as prescribing regular therapy for one or three months, for example, monthly reports to the Health cash register and others. According to the current rules, general practitioners must provide 24-hour patient care, which is physically impossible. In this regard, private structures are emerging in large cities that take over these functions, for which both the primary care practice and the patient pay. With the low standard of living and especially for the elderly, this is a significant barrier and they turn to emergency medical care centers, which are state-owned and free of charge. This causes them to be overburdened and may have to wait hours for a review. General practitioners are at the entrance of the health system and determine the movement of patients in it, expecting them to be able to make an initial decision about their health problem, as well as carry out regular consultations with other specialists. This is done by issuing a medical referral. For each individual or group general practice, the Health Insurance Fund determines this number according to an established methodology, which number is insufficient. This is usually a reason for conflict between doctor and patient, and in some cases it is the reason for changing the doctor and choosing a new one. The same applies to the directions for laboratory and imaging studies. Each practice receives a specific budget to use for these purposes. Practices usually face the imposed cash limits, which greatly hinders their freedom to operate. From June 2022 an electronic system was introduced in the country for registering examinations and issuing directions for consultations and research. This eased the work of the doctors in the practice, since the issuing of the documents is now done remotely, but at the expense of this, the phone calls on this occasion increased, which interrupts and interferes with the regular work of the doctor. Another significant problem is the home visit that general practitioners are required to carry out. Since they are not paid by the funding body (the Health Insurance Fund) and with the disappearance of the regional principle (a patient's home can be far from the doctor's practice), there has been a significant decrease in their number in recent years. Against the background of Bulgaria's aging population, which is increasingly in need of medical care, rising inflation and insufficient financing of primary care, **new opportunities for reform** in this part of the system are being discussed in the public space. In June 2022 started the electronicization of health care with the introduction of an electronic patient record, which is part of the current change in the work of primary health care. Based on this, one of the parties of the ruling coalition proposes radical solutions for future reform - abolishing the choice of a general practitioner and thereby ending the permanent relationship of a doctor with "his" patient and liquidating a patient list, giving freedom to the patient for a visit directly to a specialist without the need for a referral issued by the general practitioner, removal of capitation as a method of financing the practices, removal of the obligation for 24 hours service and transferring these responsibilities to emergency and urgent care structures. In practice, the listed proposals aim at a complete change of the previous model and the introduction of a new organization and financing of primary medical care, which will largely represent a return to the pre-2000 model.

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RESEARCH ARTICLE

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Evaluation of the Clinical Results of Using Microfluidic Channel System for Sperm Selection in IVF Cycles in Patients with Low Sperm Concentration

ABSTRACT

Objective: Microfluidic channel system (MAC), a new generation method, gives the chance to select better quality spermatozoa with lower DNA fragmentation indices. This study evaluated the treatment results in patients who underwent ICSI-ET due to the MAC technique's male factors.

Methods: Sakarya University ART Center carried out this retrospective study. Patients with 35 male factor indications were included in our study. In these patients, swim-up (SU) was used in the first of two consecutive IVF cycles, and the MAC sperm preparation technique was used in the second. Our study compared fertilization, quality embryo counts, implantation after fresh embryo transfer, pregnancy rates, fifth-day embryo, and frozen embryo numbers.

Results: Fertilization rate was higher in the MAC group than in the SU group (P=0.009). The number of 3rd and 5th Day Grade 1 embryo in the MAC group was statistically higher than in the SU group (p=0.000 for both parameters). The number of quality embryos frozen on day 5 was higher in the MAC group than in the SU group (P=0.000).

Conclusions: It is thought that MAC application does not make a statistically significant contribution on implantation and pregnancy in IVF cycles performed due to the malefactor. However, it may positively affect fertilization rate and embryo quality. In addition, we think that it increases the number of embryos frozen at the end of the cycle, and for this reason, the MAC technique may provide positive benefits to IVF treatments.

Keywords: Embryo Implantation, Infertility, Microfluidics.

Düşük Sperm Konsantrasyonu Olan Hastalarda Tüp Bebek Döngülerinde Sperm Seçiminde Mikroakışkan Kanal Sistemi Kullanmanın Klinik Sonuçlarının Değerlendirilmesi

ÖZET

Amaç: Yeni nesil bir yöntem olan mikroakışkan kanal sistemi (MAC), daha düşük DNA fragmantasyon indekslerine sahip daha kaliteli spermatozoa seçme şansı vermektedir. Bu çalışmada MAC tekniğinin erkek faktörleri nedeniyle ICSI-ET uygulanan hastalarda tedavi sonuçları değerlendirilmiştir.

Gereç ve Yöntem: Çalışmamız Sakarya Üniversitesi ART Merkezinde retrospektif olarak gerçekleştirdi. Çalışmamıza 35 erkek faktörü endikasyonu olan hastalar dahil edildi. Bu hastalarda, ardışık iki IVF döngüsünün ilkinde swim-up (SU), ikincisinde MAC sperm hazırlama tekniği kullanıldı. Çalışmamızda fertilizasyon, kaliteli embriyo sayısı,embriyo transferi sonrası implantasyon, gebelik oranları, beşinci gün embriyo sayısı ve dondurulmuş embriyo sayıları karşılaştırılmıştır.

Bulgular: Döllenme oranı MAC grubunda SU grubuna göre daha yüksekti (P=0.009). MAC grubundaki 3. ve 5. Gün Grade 1 embriyo sayısı SU grubuna göre istatistiksel olarak daha yüksekti (her iki parametre için p=0.000). 5. günde dondurulan kaliteli embriyo sayısı MAC grubunda SU grubuna göre daha yüksekti (P=0.000).

Sonuç: Erkek faktörü olan tüp bebek sikluslarında MAC uygulamasının implantasyon ve gebelik üzerine istatistiksel olarak anlamlı bir katkı sağlamadığını düşünmekteyiz.. Ancak fertilizasyon oranı ve embriyo kalitesini olumlu yönde etkileyebileceğini düşünmekteyiz. Ayrıca döngü sonunda dondurulan embriyo sayısını arttırdığını ve bu nedenle MAC tekniğinin tüp bebek tedavilerine olumlu katkı sağlayabileceğini düşünüyoruz.

Anahtar Kelimeler: Embriyo İmplantasyonu, İnfertilite, Mikroakışkan.

INTRODUCTION

Success rates in assisted reproductive techniques (ART) have increased significantly since treatment, especially in the last ten years (1). Similar to in vitro fertilization (IVF) cycles, in the success of treatment in intrauterine insemination (IUI) cycles; Factors such as female age, duration of infertility, ovarian reserve, and sperm parameters play a role (2). The importance of sperm parameters in ART treatments has been better understood by decreasing fertilization success in intra-cytoplasmic sperm injection (ICSI) procedures performed with sperm with low sperm count and motility (1). Therefore, selecting functionally normal sperm with fertilization ability is an essential need in ART treatments. Density gradient (DG) and swim-up (SU) techniques procedures are generally used as standard sperm preparation techniques in ART treatments (3). In both techniques, the centrifuge is used during sperm preparation and selection. However, centrifuges have a detrimental effect on sperm viability and can cause sperm DNA fragmentation (3). When sperms with DNA damage prepared by these methods are used in ICSI procedures, fertilization success and the possibility of obtaining good quality embryos may be adversely affected. When SU and DG methods are compared, it has been reported that a lower rate of standard chromatin structure is obtained in sperm prepared by DG method (4, 5). Although there is no consensus, using centrifuges for shorter times in the SU method is recommended. MAC, a new method developed recently for sperm selection, aims to increase high oocyte fertilization and pregnancy (6). Although DNA fragmentation has been shown to decrease in sperm selected by the MAC method, there is no clinical study showing that embryo quality and pregnancy rates increase (7, 8). While preparing sperm samples in the classical SU method, sperm are exposed to chemicals and the adverse effects of centrifugation methods, while sperm preparation in the MAC method imitates the natural sperm selection pathways in the female reproductive system (9, 10).

This retrospective cohort study aims to compare the effects of the MAC method and the SU method on the number of fertilized oocytes, quality embryos, the number of frozen embryos, and pregnancy outcomes in ART cycles due to the male factor. This study will be the first in the literature because it compares the treatment results of patients whose sperm preparation method was SU in the previous ART trial in the same patient, instead of comparing the effects of the MAC method in ART cycles with the results of different patients.

MATERIAL AND METHODS

Ethics Statement: This study was carried out retrospectively with the approval of the Sakarya University Faculty of Medicine Non-Invasive Ethics Committee dated 14.12.2020 and numbered 633.

Patients: The couples in this study consisted of patients who applied to Sakarya University Medical Faculty IVF center for treatment between 2019-2020. In our study, 35 couples who did not achieve pregnancy by using SU as the sperm preparation technique in the first trial of two consecutive ART treatments and who used the MAC method in the subsequent trial were included. Laboratory conditions, embryo culture and media used, sperm immobilization media, cumulus cell extraction media, sperm injection needles, and oocyte fixation needles (holding needles) were the same in both applications. The same embryologist performed ICSI procedures of both applications. Our study will compare the blastocyst numbers obtained on the fifth day and the blastocyst numbers frozen on the fifth day. Therefore, to obtain a sufficient number of blastocysts on the fifth day, attention was paid to the fact that the female patients included in our study were younger than 42 years old and had at least 5 MII oocytes in the ICSI procedure. When we used the MAC method for sperm preparation in male patients in previous treatments at our ART center, we experienced that a sufficient number of sperm could not be obtained in patients with a sperm count less than 1 million/ml. Therefore, we did not include male patients with a sperm count less than 1 million/ml in the study. Couples with acute or chronic diseases in both trials in ART cycles were not yet included in the study. The flow chart of the patient selection included in the study is given in Figure 1.

Sperm Preparation: Semen samples were taken by masturbation method after 2-5 days of sexual abstinence. For semen samples, sperm preparation methods were used after 15-60 minutes of liquefaction. Sperm analysis was performed according to the parameters of the world health organization (WHO) (11). Only fresh sperm samples were used in the study.

Swim-up Technique: All sperm samples were incubated for liquefaction in a 37 °C incubator for 15-60 minutes. Liquefied semen samples were then diluted 1:1 with culture medium and centrifuged at 1500 rpm for 10 minutes. After centrifugation, the supernatant was removed, 1 ml of fresh medium was carefully transferred onto the sperm pellet collected at the bottom, and it was incubated for 1 hour (37 °C, 6% CO2), kept tilted at 45 degrees. After incubation, the supernatant is collected in a sterile tube. Thus, the semen sample is ready for ICSI processing.

MAC Technique: Using a sterile micropipette, 13 µL of sperm sorting solution was added to the microfluidic chip inlet port, and then 2 µL of liquefied sperm sample was slowly added to the same port.

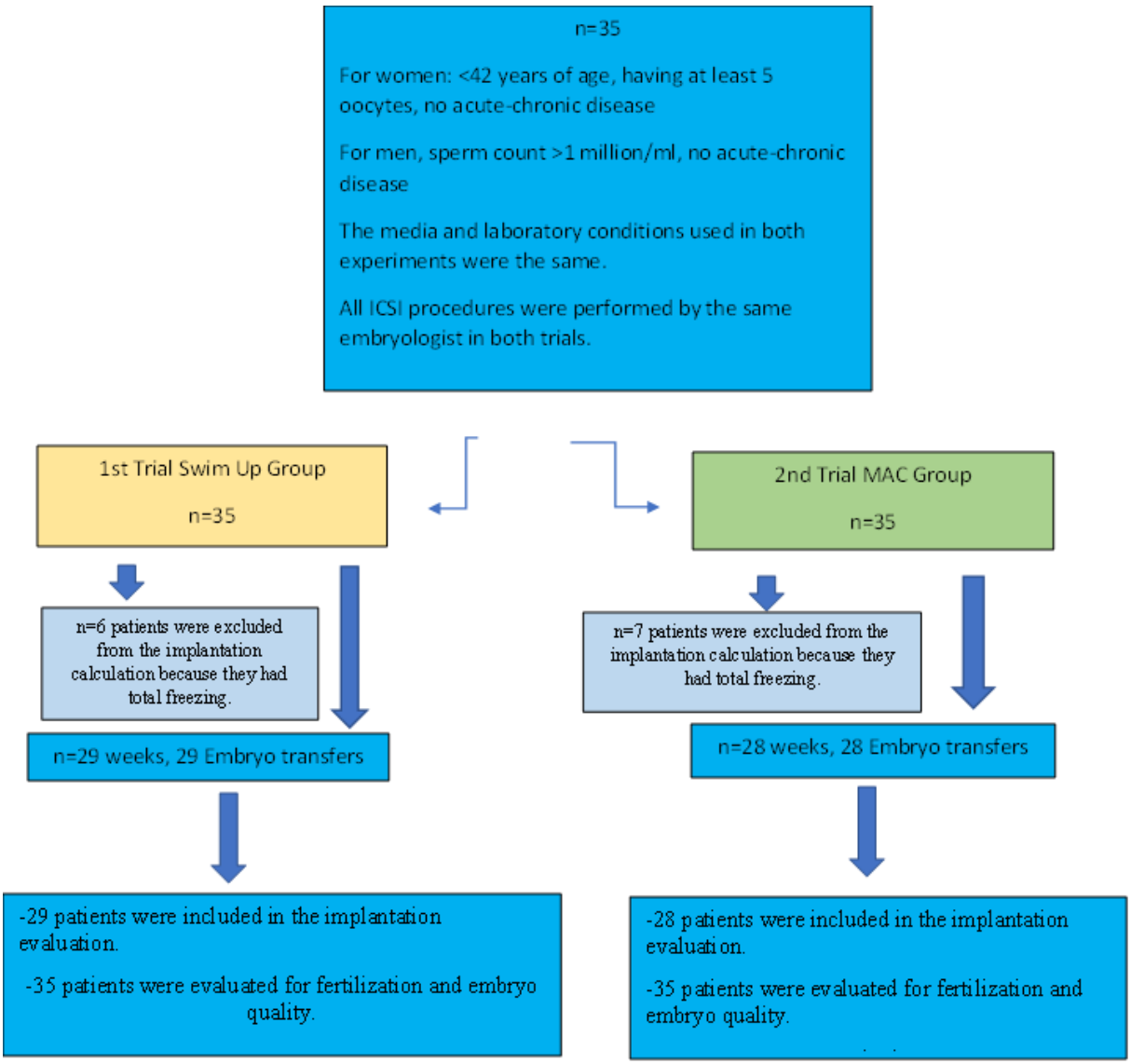

Figure 1. Patient selection flowchart.

Then, approximately 2 µL of mineral oil was added to the outlet port to protect the sperm cells against evaporation. For the same purpose, the inlet port will be covered with mineral oil and incubated at 37 degrees for about 30 minutes. After incubation, sperm cells containing solution from the outlet port under the mineral oil were examined under an inverted microscope. Sperm cells are ready for the ICSI procedure.

Controlled Ovarian Hyperstimulation and ICSI Procedure: Antagonist protocol (Cetrotide; Serono) was used as a method of controlled ovarian stimulation. Gonadotropin dose was determined according to the patients' ovarian reserve and response to the gonadotropins used. Human chorionic gonadotropin (HCG) injection was administered when the mean diameter of at least two follicles reached 17 mm. Oocytes were collected under general anesthesia by transvaginal-USG 36 hours after hCG injection (6). A single embryologist performed the ICSI procedure. Prepared sperm samples were transferred to a sterile culture dish with polyvinylpyrrolidone (PVP), ICSI, and ICSI dishes coated with sperm pool mineral oil. ICSI procedure was performed with the sperms with the best motility and morphology under the appropriate objective. After ICSI, oocytes were transferred into the culture medium, and fertilization and embryo follow-up were performed(12).

Embryo Evaluation: Fertilization control was performed according to the presence of pronucleus 16-18 hours after the ICSI procedure. Fertilized oocytes were incubated in a single-stage culture medium. According to previous studies, embryos were graded on the third day for their quality(13). Embryo culture was continued until the fifth day. The transfer status and embryos to be frozen were made according to blastocyst score grading(14).

Clinical Results: In the study, we compared the fertilization rates between the two cycles, the

number of good quality embryos on Day 3 and Day 5, the number of quality embryos frozen, and pregnancy outcomes. We compared the clinical pregnancy rates by comparing the patients who achieved pregnancy due to transfer and all the patients.

Statistical Analysis: Statistical analyzes were performed using the SPSS 24.0 package program (SPSS Inc. and Lead Tech. Inc. Chicago. USA). The Kolmogorow-Simirnow test was used for the normal distribution of the data. In our study, SU and MAC techniques, sperm preparation techniques, were applied to the same patient pairs at different time intervals. Since two sperm preparation techniques were compared, dependent groups with normal distribution were compared using the Paired-sample test. All results are presented as Mean±SD. Results with $p<0.05$ were considered significant.

RESULTS

Thirty-five patients who underwent IVF due to the male factors were included in our study. We compared the contributions of the SU technique as a sperm preparation method in the first trial and the MAC technique as a sperm preparation method in the subsequent trials to IVF treatments.

The mean age of the women included in the study was 32.01±0.96 years; the mean age of men was found to be 35.02±0.98 years. The mean BMI was found to be 24.7±5.7 in women and 28.1±3 in men. There was no statistically significant difference between the total amount of gonadotropin used in the treatment process, sperm concentration, sperm morphology, total sperm motility, and progressive sperm motility in terms of both treatments ($p>0.05$) TableI.

Table I. Comparison of total gonadotropin and sperm parameters used in two consecutive trials in the study.

Parameters	Swim-up	MAC	*P* value
Total Gonadotropin	2405±73	2556,41±69	*P*=0.181
Sperm concentration	32.5±4.3	33.1±4.1	*P*=0.872
Total sperm movement	58.3±3	62.1±2.4	*P*=0.346
Progressive sperm movement	45.3±2.6	46.2±2.1	*P*=0.811
Sperm morphology (%)	1.2±0.2	1.1±0.4	*P*=0.121

Analysis was performed with the paired sample test ($p<0.05$ was considered statistically significant).

In our study, comparisons of total oocytes, MII oocytes, fertilized oocytes, third and fifth-day quality embryo counts, third and fifth-day frozen quality embryo counts of two consecutive trials are presented in Figure 2. There was no statistically significant difference between the numbers of total oocytes, MII oocytes, and embryos frozen on day three collected between the two groups ($p>0.05$). Significant differences were observed between the number of fertilized oocytes, the number of good quality embryos on the third day, the number of good quality embryos on the fifth day, and the number of frozen good quality embryos on the fifth day in the direction of the MAC technique (Figure 2).

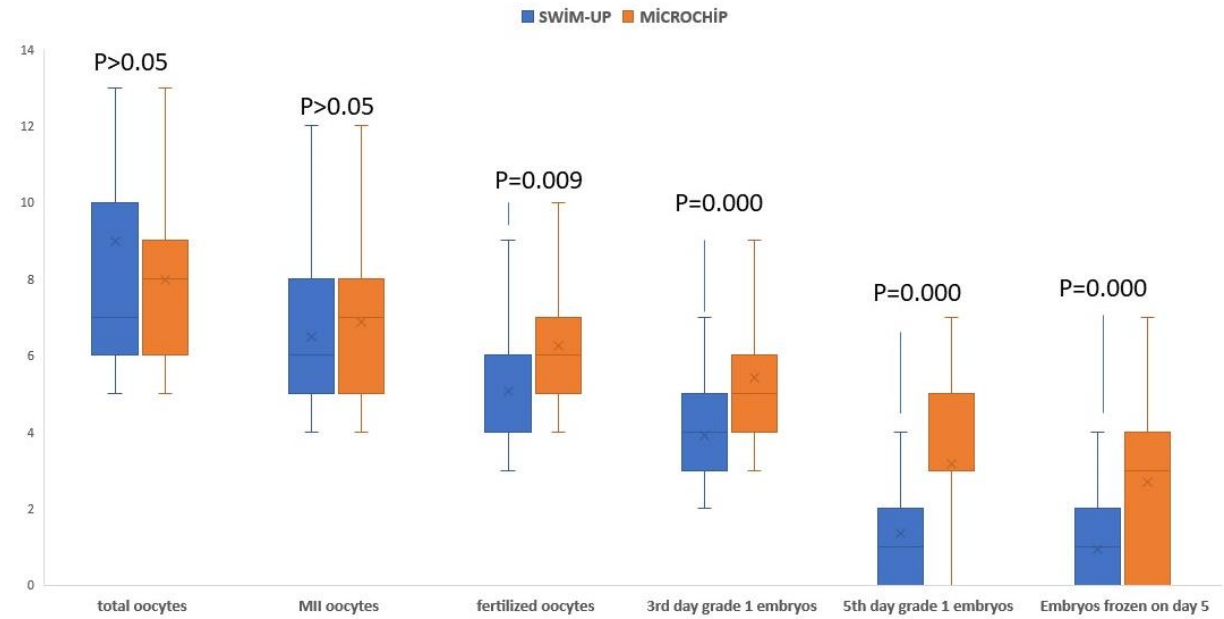

Figure 2. Comparison of oocyte and embryo values between groups.
There was no difference between the collected oocyte and MII oocyte numbers between the two groups. There were significant differences between the numbers of fertilized oocytes, 3rd and 5th day good quality embryos and 5th day frozen good quality embryos. Analysis was performed with the paired sample test ($p<0.05$ was considered statistically significant).

Since all embryos were frozen without embryo transfer in 6 patients in the SU group and seven patients in the MAC group, these patients were not included in the implantation and pregnancy evaluations. Implantation positivity was observed in 8 of 29 cases in the SU group. However, although pregnancy occurred in only one of them, it resulted in miscarriage in the following weeks. In the Mac method, implantation positivity was seen in 9 out of 28 cases. Among these cases, in cases prepared with microchip methods, pregnancy was positive in 6 of them, and pregnancy was observed in three. There was no statistically significant difference between implantation and pregnancy rates between the groups (P>0.05).

DISCUSSION

New technological developments have influenced ART. One of these new techniques is the MAC method, which imitates the physiological and biochemical environment of sperm and provides more opportunities for natural physiological sperm selection (15). In our study, we compared our patients' laboratory and clinical results whose sperm preparation method was SU in the first of two consecutive trials and whose sperm preparation method was MAC in the second trial. Different types of micro-chip types are used in ART treatments by the characteristics of sperm, such as motility and morphology. Our study used the type of sperm isolator according to sperm motility (16). The type used for the motility of the microchip is in principle similar to the SU technique in sperm separation. However, the microchip features such as long narrow channels and fluid flow differ from the SU method (17).

The contribution of microchip application to clinical outcomes is still not demonstrated (16). For these reasons, in our study, we examined the contribution of the microchip to implantation and clinical pregnancy outcomes in ART treatments, as well as to fertilization, obtaining quality embryos, the number of embryos on the fifth day, and the number of embryos frozen on the fifth day. Achieving a high fertilization rate and increasing the number of quality embryos in ART treatments are not dependent on a single cause or condition. Sperm preparation methods and good sperm isolation are important among the conditions that directly affect fertilization and obtaining quality embryos. There are very few studies investigating the effects of new technologies on embryo quality (6, 18, 19). It has been reported that sperm selection with the help of an inverted microscope causes an increase in the number of embryos of higher quality and ongoing pregnancies compared to the classical ICSI method (20). In our study, when we compared the MAC method, which is a recent innovation in sperm preparation method, and the SU method, which has been used conventionally for a long time; We observed that fertilization rates and the number of quality embryos on the third and fifth days increased with the MAC method. However, we observed no difference in implantation and clinical pregnancy rates. Similar to our study, only two studies in the literature compare and evaluate the contribution of MAC and SU methods to ART treatments (18, 19). Similar to our study, in one of these studies; It has been reported that the MAC method increases the number of quality embryos compared to the SU method and does not affect pregnancy outcomes (19). Unlike our study, it was reported that the Microchip method did not contribute to fertilization rates in this study (19). However, incomplete information was given about the days of the transferred embryos in the same study (19). In the other study, MAC and SU methods were compared, and they reported that they did not find any difference between laboratory and clinical findings in these two applications (18). However, in this study, it is understood that different periods are used in the sperm preparation process with the MAC method than the MAC manufacturer's working procedure (18). This may have affected the results of the study.

The current method for selecting high-quality embryos in embryology laboratories in vitro fertilization and embryo transfer (IVF-ET) practice is based on the quality criteria of day three embryos (21). This criterion is generally based on the number of cells, symmetry, and degree of fragmentation in the day three embryo after fertilization (22). According to this criterion, day three embryos containing eight blastomeres are preferably selected for fresh embryo transfers because a high rate of blastocyst formation and clinical pregnancy were associated (22). The advantages of this situation are that day five embryos have a lower risk of aneuploidy and a higher physiological synchronization with the endometrium (23, 24). In addition, the transfer of embryos at the blastocyst stage is hypothesized to prevent early exposure of the embryo to super physiological levels of estrogen and progesterone resulting from controlled ovarian stimulation(25). Besides these advantages, there are differences between the number of embryos obtained at the cleavage stage and between advancing the embryo culture and extending it to the blastocyst stage (25). Therefore, fewer embryos can be obtained because of the number of embryos remaining after transfer or in cycles extended to the blastocyst stage for all milled embryos since not every embryo can develop until the blastocyst stage. Therefore, there is still a persistent debate about whether to delay the transfer or advance embryo culture to the blastocyst stage (18). In our study, the blastocyst embryos obtained on the fifth day were higher in the MAC group than in the SU group. In a similar study, no significant difference was found between the embryos obtained at the blastocyst stage between the MAC and SU groups (18). Yetkinel et al.(19) obtained more quality embryos with the

MAC method than the SU method, but they did not give clear information about the embryo days. We think that the use of MAC will help select quality sperm and reduce the hesitations of advancing embryo culture to the blastocyst stage after fertilization. Due to the high embryo survival rates of the cryopreservation method in recent years, vitrification has been preferred over the slow-freezing method in embryo freezing-thawing processes (26). Fernandez-shaw et al.(25) reported that cumulative pregnancy rates in vitrified freeze-thaw cycles were significantly higher when blastocyst embryos were used than in cleavage stage embryos. When embryo transfer from fresh and thawed IVF cycles is evaluated, it has been reported that cumulative pregnancy rates are more successful in cases advanced to the blastocyst stage (23). In our study, after day 5 embryo transfer in fresh IVF cycles, more Grade 1 embryo were obtained in the MAC group than in the SU group. The resulting embryos were frozen for use in subsequent freeze-thaw cycles. Similar to our study, studies are reporting that more embryos are frozen with the MAC technique than with the SU technique after transfer (19). Unlike our study, there is also a study where it was reported that there was no difference between the number of frozen Day 5 embryos in the MAC and SU groups (18). Our study observed that the MAC method provided more Day 5 embryos to be used in Freeze-thaw cycles. Thus, we think that obtaining more quality embryos in ET with MAC or milling-all cycles may be beneficial in ART cycles.

In a study on sperm selection, it was reported that the importance of extending embryo development to the blastocyst stage should not be underestimated, as paternal effects are more decisive on the third day and beyond (24). The MAC method is thought to differentiate spermatozoa with higher DNA integrity (27). We think that this situation increases the blastocyst retrieval rates. In addition, it has been reported that the MAC method may be helpful to reduce the miscarriage rates, which are thought to occur due to high sperm DNA fragmentation (28, 29). In line with the information, we obtained in our study, we recommend the MAC method to infertile couples with male factor indications who have clinical difficulties in obtaining quality embryos. Although a very high pregnancy rate could not be obtained in the MAC method trials, we saw that the number of quality embryos obtained in the cycles using MAC increased considerably. Freezing of quality embryos has positive effects in ART trials. We think that frozen embryos will increase the number of pregnancies with appropriate endometrium and luteal phase support in obtaining pregnancy in later trials.

CONCLUSION

Our study observed that the MAC technique did not positively contribute to implantation and pregnancy outcomes in ART cycles performed due to the male factors, but it increased the fertilization rate, the number of quality embryos, and the number of frozen fifth-day embryos after ICSI. The higher number of frozen embryos will allow infertile couples to achieve pregnancy when the subsequent cycles are considered.

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RESEARCH ARTICLE

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Is the *AURKB* Gene Involved in AML Cell Proliferation Since It is Targeted by miR-34a-5p and let-7b-5p?

ABSTRACT

Objective: The production of normal blood cells in the bone marrow is interrupted in AML, which is characterized by the proliferation and accumulation of leukemic blasts. Therefore, patients experience anemia and thrombocytopenia. When gene expression of Aurora kinases, which is reported to be highly expressed in AML, decreases, it may be possible to alleviate the clinical findings in AML. In this study, it was aimed to examine the relationship of *Aurora kinase B (AURKB)* with important miRNAs that have the potential to regulate gene expression

Method: HL60 and NB4 cells were transfected with important tumor suppressor miRNAs miR-34a-5p and let-7b-5p mimics. Then, its effects on proliferation were examined with WST-8 technique and its effects on *AURKB* gene expression were examined with qRT-PCR.

Results: It was determined that these miRNAs negatively regulated proliferation in both AML cell lines and downregulated the expression level of the *AURKB* gene in the miRNA transfected group compared to the control group.

Conclusion: In conclusion, it was determined that miR-34a-5p and let-7b-5p could regulate *AURKB* expression in AML cells. Therefore, it was thought that these miRNAs may have an important potential as a therapeutic biomarker in preventing excessive cell division and poor prognosis in AML.

Keywords: AML, *AURKB*, miR-34a-5p, let-7b-5p.

AURKB Geni miR-34a-5p ve let-7b-5p Tarafından Hedeflenerek AML Hücre Proliferasyonunda Rol Oynayabilir mi?

ÖZET

Amaç: Lösemik blastların çoğalarak birikimiyle karakterize olan AML'de kemik iliğindeki normal kan hücrelerinin üretimi sekteye uğradığından hastalar anemi ve trombositopeni sorunu yaşamaktadır. AML'de yüksek oranda eksprese edildiği bildirilen Aurora kinazların gen ekspresyonu azaldığında AML'deki klinik bulguları hafifletmesi mümkün olabilir. Bu çalışmada, *Aurora kinase B (AURKB)* ile protein ekspresyonunu düzenleme potansiyeli bulunan önemli miRNA'ların ilişkisinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: HL60 ve NB4 hücreleri, önemli tümör supresör miRNA'lardan olan miR-34a-5p ve let-7b-5p mimikleri ile transfekte edilmiştir. Ardından bu miRNA'ların proliferasyona etkisi WST-8 tekniğiyle, *AURKB* gen ifade değişimi üzerindeki etkisi ise qRT-PZR ile incelenmiştir.

Bulgular: Her iki AML hücre hattında da bu miRNA'ların proliferasyonu negatif yönde regüle ettiği ve kontrol grubuna kıyasla miRNA transfekte edilen grupta *AURKB* geninin ifade seviyesini downregüle ettikleri belirlenmiştir.

Sonuç: Sonuç olarak miR-34a-5p ve let-7b-5p'nin AML hücrelerinde *AURKB* ifadesini düzenleyebileceği tespit edilmiştir. Bu nedenle, AML'deki aşırı hücre bölünmesi ve kötü prognozun engellenebilmesinde bu miRNA'ların terapötik bir biyomarker olarak önemli bir potansiyeli olabileceği düşünülmüştür.

Anahtar Kelimeler: AML, *AURKB*, miR-34a-5p, let-7b-5p.

INTRODUCTION

Acute myeloid leukemia (AML) is an important hematopoietic disease in which clonal proliferation of myeloid cells is seen due to various genetic and epigenetic alterations (1). Even though several inductions and remission therapies are now available for AML, survival rates are still poor. So, it is important to identify new diagnostic and therapeutic biomarkers for AML, which constitutes the vast majority of leukemias (2).

Aurora is a family of serine/threonine kinases whose importance has been reported in the stages of chromosome distribution and cytokinesis in cell division (3, 4). Studies are needed to understand the mechanisms by which members of this kinase family contribute to cancer processes. It has been reported that the expression of Aurora A and *AURKB* is increased in a wide variety of solid tumors compared to normal tissue, and elevated Aurora expression levels have been reported in association with tumor recurrence and the advanced stages of the disease (5, 6). In the literature, abnormally increased expression of Aurora A and B kinases has been reported in hematological malignancies, including acute myeloid leukemia. It has been reported that ZM447439, which is an inhibitor of AuroraA and B kinase, increases the tendency to apoptosis by reducing cell growth in leukemia cells (7). The MLN8054 is another Aurora kinase inhibitor that has been reported to suppress tumorigenesis in lung cancer xenograft experiments (8).

Despite the use of all these Aurora kinase inhibitors, the need for more effective agents against Aurora kinases brings to mind microRNAs (miRNAs). MiRNAs are small non-coding RNAs that regulate gene expression. It is possible to group miRNAs according to the characteristics of the gene that are silenced via miRNAs. For example, if a miRNA plays a role by suppressing an oncogenic gene in the regulation of gene expression, it is called tumor suppressor miRNA (Ts-miR), whereas if it plays a role by suppressing a tumor suppressor gene, it is called oncogenic miRNA (onco-miR). MiRNAs that are important for many cancers, including AML, have been reported in the literature. In AML, miR-9 (9) and miR-139 (10) are reported as Ts-miR, while miR-155 (11) is reported as an onco-miR. miR-34a-5p and let-7b-5p, whose in vitro function was investigated in this study using AML cell lines, are among the important miRNAs reported as Ts-miR for many cancers in the literature. Like Aurora kinase inhibitors reported in the literature, it may be possible to suppress gene expression by targeting Aurora kinases through miRNAs and thus reduce their contribution to cancer formation. Based on this, in our study, the relationship between *AURKB* gene and the 2 important Ts-miRs was investigated in HL60 and NB4 AML cell lines.

MATERIAL AND METHODS

In Silico Detection of the *AURKB* Gene as a Target of miR-34a-5p and let-7b-5p: The expression of *AURKB* in various cytogenetic anomalies associated with AML was determined by the bloodspot tool (12). The hub proteins directly related to the *AURKB* gene were found using the Enrichr database. The relationship between *AURKB* and hub proteins was visualized by String (13). The *AURKB* gene has been shown to be highly expressed in AML. Thus TsmiRs whose expression is reduced in AML were identified first. The TsmiR miRNAs associated with AML in the literature were identified by reviewing the (14, 15) studies. The miRNet tool was used to determine whether the identified TsmiRs could potentially target the *AURKB* gene (16). miRDB (17), Targetscan (18) miRWalk (19) were utilized for confirmation. Following a review of the literature, 2 miRNA was selected for in vitro study and the complementarity of miR-34a-5p and let-7b-5p with *AURKB* 3'UTR is checked via STarMirDB tool (20).

HL60 and NB4 Cell Culture and Transfection of miRNA Mimics: HL60 and NB4 AML cell lines were used in this study. Cells were seeded and cultured under the 37°C and 5% CO2 incubator condition and 10% FBS-1% antibiotic added RPMI-1640 medium condition. According to the manufacturer's procedure, transfection of 30 pmol miR-34a-5p, let-7b-5p and non-targeting (nt) control) mimics (Thermo Fisher Sci. Inc.) were performed on cells using the lipofectamine-mediated method (Lipofectamin 2000, Thermo Fisher Sci. Inc.).

miR-34a-5p and let-7b-5p Effect on Cell Proliferation: The WST-8 technique, which provides information about viability colorimetrically, was used to examine whether the selected miRNAs have an effect on cell proliferation. For this purpose, $5x10^3$ cells were inoculated on a culture plate (96-well plate) in three wells for each miRNA mimics and nt control mimic. Using the CVDK-8 kit (EcoTech Biotechnology) protocol cell proliferation was determined by measuring absorbance at 450 nm with the MultiScan FC microplate reader (Thermo) at 48 and 96 hours. Cells were also observed with an inverted fluorescent microscope (Nikon ECLIPSE 80i).

RNA Extraction: After transfection, total RNAs of the study group and control group AML cells were extracted according to the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as stated in the procedure instruction. RNA purity and concentrations were determined spectrophotometrically with NanoDrop 2000 device (Thermo Fisher Scientific, Madrid, Spain).

qRT-PCR Process for miRNA Transfection Verification: For the determination of miR-34a-5p and let-7b-5p quantify in miRNA

mimic transfected cells, selected miRNA and RNU43 (control miRNA) specific TaqMan primer-probes were commercially purchased. cDNA was synthesized with a total of 30 ng RNA using TaqMan primers (Thermo Fisher) and TaqMan MicroRNA reverse transcriptase kit (Applied Bio., Foster City, CA, USA). Then, qRT-PCR was performed with TaqMan probes (Thermo Fisher) and TaqMan Universal Master Mix (Thermo Fisher).

Expression Analysis of *AURKB* Kinase: cDNA synthesis (SCRIPT kit, Jena Bioscience) was performed using RNAs extracted from miRNA mimic and nt mimic transfected cells. For this purpose, a total of 1000 ng RNA was used, and then expression analysis was performed by a qRT-PCR technique using SybrMaster reagent (Jena Bioscience) and F/ R primers of the *AURKB* gene (Forward-5′-ATTGCTGACTTCGGCTGGT-3′, Reverse-5′-GTCCAGGGTGCCACACAT-3′) (21). Forward-5'-GCCTCGCCTTTGCCGATC-3' and Reverse-5'-CCCACGATGGAGGGGAAG-3' primers specific to the *β-Actin* gene were used as a housekeeping control (22).

Statistical Analysis: The experiments were performed in duplicate for qRT-PCR analysis and for in triplicate for WST-8 assay. The relative quantitation method ($2^{-\Delta\Delta Ct}$ method) was used for the evaluation of the expression levels. The data were analyzed by SPSS 28 program and stated as mean ± standard deviation (SD). $p<0.05$ value was considered statistically significant and the figures were drafted using GraphPad Prism 9.3 software.

RESULT

In Silico Analysis Data: The expression of *AURKB* in various cytogenetic anomalies associated with AML is shown in Figure 1. As a result of PPI analysis, it was determined that *AURKB* was directly related to 9 hub proteins (Figure 2). Following a review of the literature, 30 miRNAs were identified as acting as TsmiR in AML (Figure 3). Six of these miRNAs were determined to have the potential to target *AURKB* (Figure 4 and Figure 5). After the confirmation of the findings with miRWalk, miRDB, and Targetscan, as well as a deeper investigation of the literature, miR-34a-5p, and let-7b-5p, which are assumed to be more connected to *AURKB*, were used in an in vitro study. The complementarity of miR-34a-5p, let-7b-5p with *AURKB* 3'UTR is shown in Figure 6.

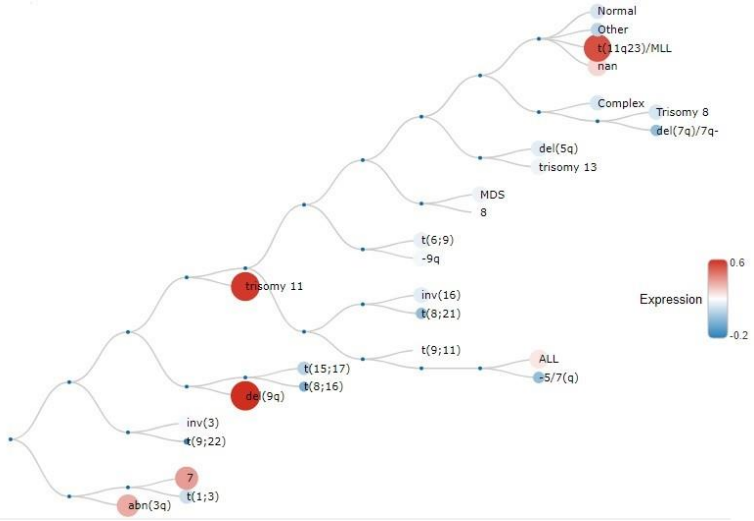

Figure 1. The expression states of the *AURKB* gene in various cytogenetic abnormality groups. Data was obtained using the 'bloodspot' tool (37). 'BloodPool: AML samples versus normal' was selected as the dataset. The selected dataset includes GSE13159, GSE15434, GSE61804, GSE14468 and TCGA.

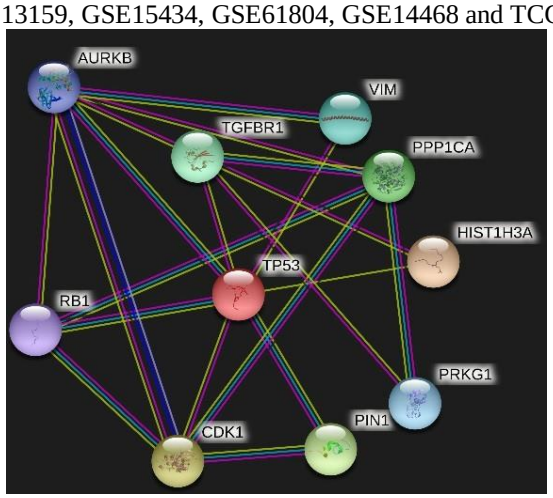

Figure 2. The relation between *AURKB* gene and its 9 hub proteins. The hub proteins were obtained using enrichr. *AURKB* and the hub genes' association was created via String (38).

Figure 3. AML associated 30 TsmiRs (14, 15).

Figure 4. *AURKB* and potantial target miRNAs acording to miRNet (39). Six of the 46 miRNAs contained here have been reported as TsmiR in AML. The six miRNAs are shown in yellow.

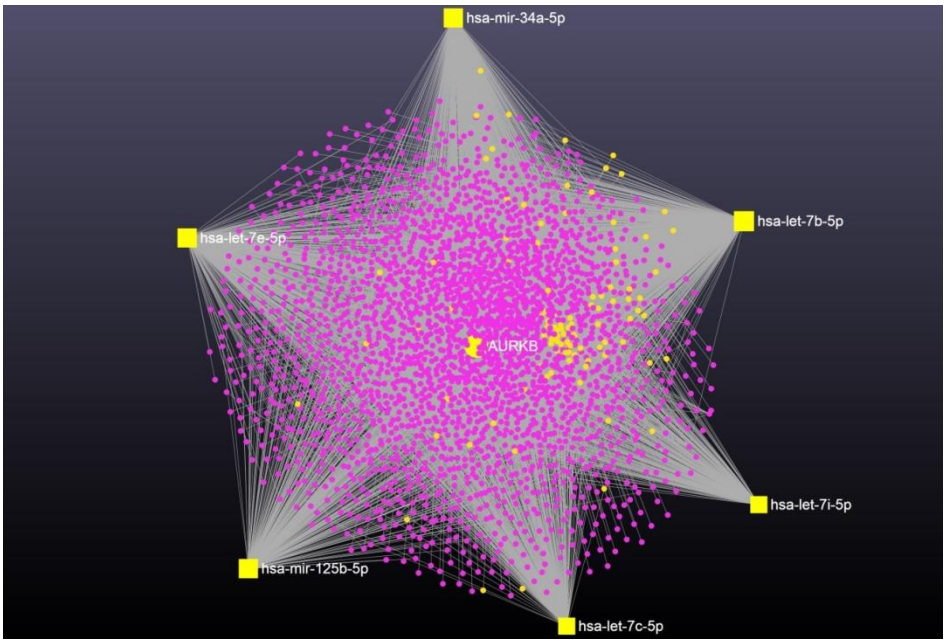

Figure 5. The connections of miRNAs and genes in leukemia. The six TsmiRs may regulate the expression of hundreds of genes, including *AURKB* (39).

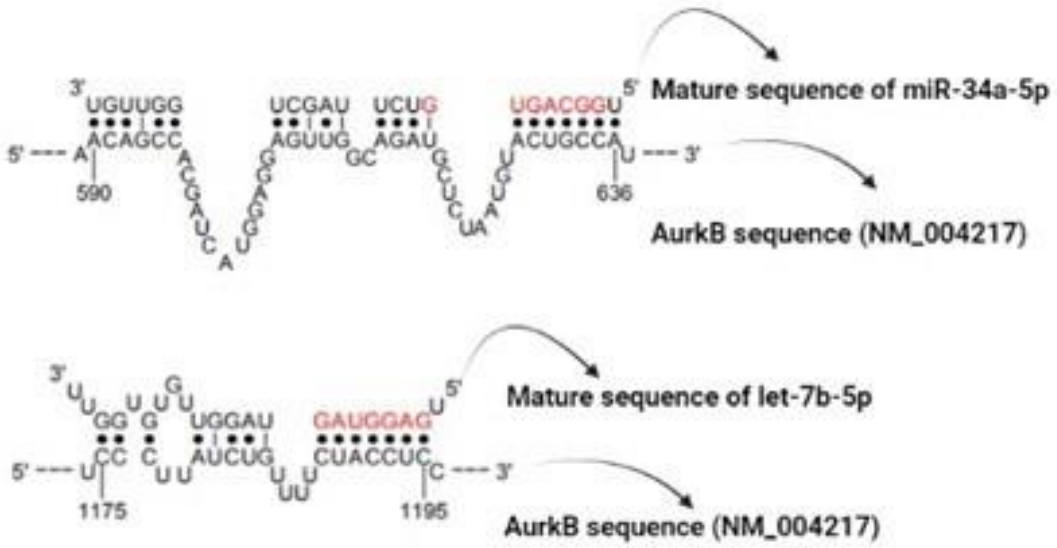

Figure 6. *AURKB* 3'UTR region complementarity with selected miRNAs (40).

Selected TsmiRs Repress the AML Cell Proliferation: In both HL60 and NB4 cells, 2 selected miRNAs were found to have a statistically significant effect on reducing cell proliferation in the study group compared to the control group (miR-34a-5p mimic transfected HL60 cell proliferation at 48 hour p<0,001, fold change= 1,27; at 96 hour p<0,001, fold change= 1,3), (miR-34a-5p mimic transfected NB4 cell proliferation at 48 hour p<0,05, fold change= 1,23; at 96 hour p<0,001, fold change= 1,41), (let-7b-5p mimic transfected HL60 cell proliferation at 48 hour p<0,001, fold change=1,3; at 96 hour p<0,001, fold change= 1,37) and (let-7b-5p mimic transfected NB4 cell proliferation at 48 hour p<0,05, fold change= 1,1; at 96 hour: p<0,001, fold change= 1,2) (Figure 7-8).

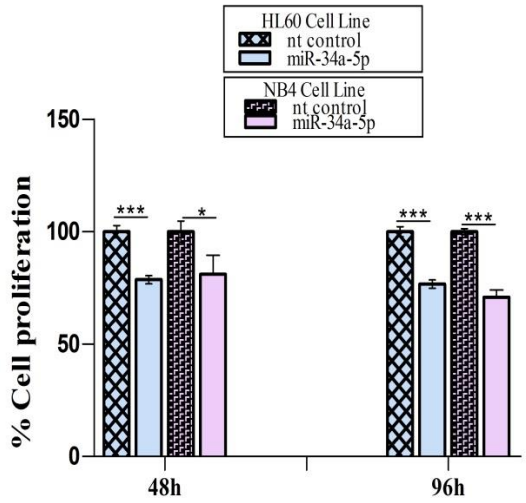

Figure 7. Effect of miR-34a-5p on cell proliferation (*p<0,05; ***p<0,001).

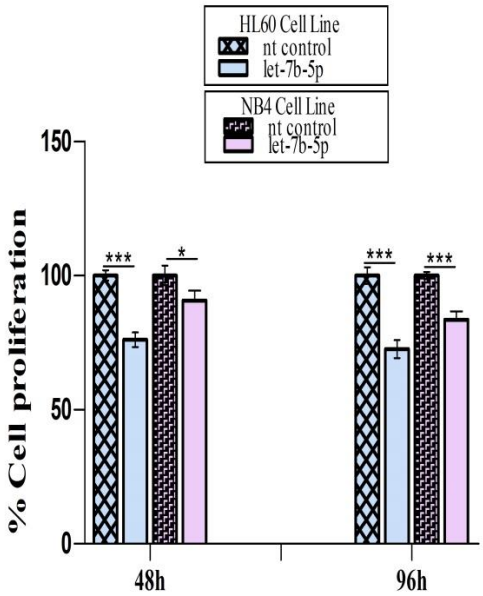

Figure 8. Effect of let-7b-5p on cell proliferation (*p<0,05; ***p<0,001).

***AURKB* Gene Expression was Negatively Regulated via Selected TsmiRs:** *AURKB* gene was found to be statistically less expressed in HL60 and NB4 cells transfected with selected miRNA mimics compared to control groups (miR-34a-5p mimic transfected HL60 cells p<0,05 ; fold change= 4); (let-7b-5p mimic transfected HL60 cells p<0,01 ; fold change= 2,32), (miR-34a-5p mimic transfected NB4 cells p<0,01 ; fold change= 1,6) and (let-7b-5p mimic transfected NB4 cells p<0,01 ; fold change= 5) (Figure 9-10). This result indicates that miR-34a-5p and let-7b-5p may play a role in regulating the expression of the *AURKB* gene as a negative regulators in HL60 and NB4 cells.

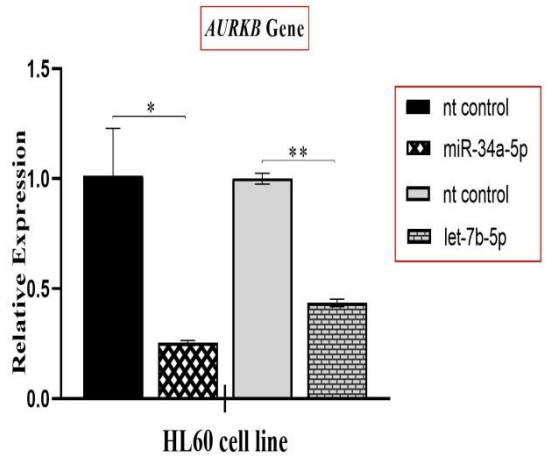

Figure 9. The expression level of *AURKB* gene in HL60 cells transfected with selected miRNA mimic and nt mimic (*p<0,05; **p<0,01).

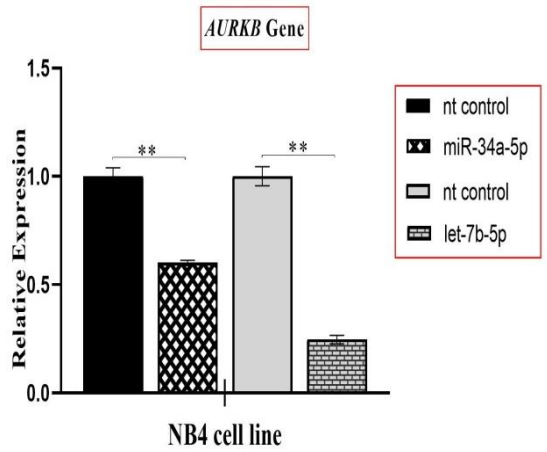

Figure 10. The expression level of *AURKB* gene in NB4 cells transfected with selected miRNA mimic and nt mimic(**p<0,01).

DISCUSSION

Acute myeloid leukemia (AML) is a hematological malignancy in which a boost in myeloid stem cells is seen in the bone marrow under the influence of various genetic-epigenetic and/or environmental factors (1). Although a wide variety of cytogenetic and molecular genetic changes such as t(8;21), inv(16), t(15;17), and *FLT3* mutation are used in the rapid diagnosis and prognosis of AML, there is still a need to identify novel molecules that may be useful for early diagnosis and treatment (23). As an example of the research used for AML and the treatments on which studies are still ongoing; immunotherapy, development of gene-specific inhibitors against various gene mutations (*FLT3*, *TP53*, *IDH1/2*, checkpoint inhibitors, etc), antibody-based studies, CART cells treatments could be shown (24). One of the inhibitor treatment is using Aurora kinase inhibitor in AML. Aurora kinases, which are important molecules in cellular mitosis and cytokinesis, have been reported to have increased expression levels in many cancers (25). Since problems with mitosis in cell division are known to increase genomic instability, it is essential to control the amount of Aurora kinase protein molecules which involved in mitosis (26). Therefore, several inhibitors have been developed for members of this protein kinase family and some of them have received FDA approval (27). In t(8;21) AML cells, inhibition of the cell cycle and proliferation has been reported through inhibition of *AURKA* (inhibitor Alisertib) and *AURKB* (inhibitör Barasertib) (28). In addition, various molecules such as ZM447439, AZD2811 and AZD1152 have been developed as Aurora kinase inhibitors in leukemias including AML (29) and It has been proposed that utilizing these compounds in combination with certain chemotherapeutics can boost their efficiency and also it has been suggested that use of the FDA-approved *FLT3* inhibitor "Gilteritinib" with *AURKB* inhibitors may be beneficial prior to relapse in AML (30). It has been reported in the literature that *AURKB* is a more essential protein than other Aurora kinases for the proliferation and survival of AML cells (31). Due to this reason, in our study, we focused on alternative *AURKB* targeting strategies. In recent years, studies have brought up the idea of miRNA-mediated suppression of the expression of Aurora kinase genes, and miRNA researches are increasing as an alternative to Aurora kinase inhibitors. MiR-34a-5p and let-7b-5p, whose relationship with *AURKB* we examined in this study, are very important TsmiRs known to play a role in suppressing tumor growth and progression in many cancer types including AML (32, 33). It is expected that by increasing the amount of these TsmiRs in the cell, they can suppress their targeted oncogenes more effectively, thereby reducing processes such as cell proliferation, metastasis and cancer progression. To the best of our knowledge, there is no study investigating the relationship between *AURKB* and miR-34a-5p and let-7b-5p in AML, and also there are limited studies on this subject in other cancers as well.. It has been reported in the literature that in some cancers, due to decreased levels of some miRNAs including miR-34a, the gene expression of the *FOX* family up-regulated, which indirectly increases the expression of genes such as *CCNB1*, *AURKB*, *MYC*, leading to poor prognosis (34). In a study investigating the let-7b/*AURKB* relationship in HeLa and HCT-116 cells, it was reported that increased *AURKB* expression is seen in many cancers and this situation causes poor prognosis in patients. In the same study, let-7b was found to contribute to the reduction of genomic instability by targeting *AURKB*, which is an important protein for the mitosis stage (21). In addition, there are some studies reporting that let-7b reduces drug resistance of gastric cancer cells (35) and it could be

associated with resistance in K562 chronic leukemia cells (36) by targeting *AURKB*.

In our study first, we conducted a literature review and used various in silico tools to identify miRNAs that could regulate *AURKB* expression. Following in vitro study, we revealed that miR-34a-5p and let-7b-5p miRNAs play a role in decreasing HL60 and NB4 cell proliferation and suppressing *AURKB* expression statistically significantly. Among the limitations of our study, we examined the effect of miR-34a-5p and let-7b-5p only on AML cell proliferation and on *AURKB* gene expression in AML cells. Another limitation is that we have not yet confirmed the accuracy of our data in AML patients. Therefore, this study must be investigated in terms of its effect on protein and miRNA-gene interaction using advanced techniques in both AML cell lines and bone marrow samples from individuals with AML.

CONCLUSION

Mortality rates are still quite high in AML, which is a rare type of cancer compared to many diseases. Therefore, it may be a good alternative to discover miRNA-Aurora kinases relationships both for early diagnosis and for the development of new treatments.

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RESEARCH ARTICLE

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The Role of Metacognition in the Emergence of Anger and Aggression in Patients with Alcohol Use Disorder

ABSTRACT

Objective: In this study, we aimed to investigate metacognitive functions, anger and aggression and the relationship in patients with alcohol dependence. It is to have information about which metacognitive beliefs plays a role in the emergence of anger and aggression in people with alcohol dependence.

Method: The patient group diagnosed with Alcohol Use Disorder (AUD) according to DSM-5 (n = 72) and the control group without any psychiatric diagnosis (n = 71) were included in the study. Sociodemographic data form, Alcohol Use Disorders Identification Test (AUDIT), Metacognition Questionnaire (MCQ-30), Trait Anger and Anger Expression Scale (STAXI) and Buss-Perry's Aggression Questionnaire (AQ) were used.

Results: In the AUD group, the MCQ-30 total, STAXI trait anger, anger out and anger in, and AQ total scores were found to be significantly higher than the control group ($p<0,001$, $p<0,001$, $p=0,001$, $p=0,001$ and $p<0,001$, respectively). When comparing the correlation coefficients of MCQ-30 and other scales between the groups, a difference was found between the AUD group, trait anger and physical aggression ($z=2,035$; $p=0,042$ and $z=2,120$; $p=0,034$, respectively). As a result of the regression analysis performed in our study, it was found that the need to control thoughts is the most metacognitive beliefs that predicts aggression in people with AUD($\beta=0.567$,$t(66)= 4,034$, $p<0.001$,$pr2=0.20$).

Conclusion: Metacognitive beliefs are highly affected in people with AUD and they cause more anger and aggression. The need to control thoughts plays an important role in the emergence of anger.

Keywords: Alcohol Dependence, Metacognition, Anger, Aggression.

Alkol Kullanım Bozukluğu Olan Hastalarda Öfke ve Agresyonun Ortaya Çıkışında Üstbilişin Rolü

ÖZET

Amaç: Bu çalışmada alkol bağımlılığı olan hastalarda üstbiliş işlevleri ve üstbilişin öfke ve agresyon ile ilişkisi araştırılmıştır. Alkol bağımlılığı olan hastalarda öfke ve agresyonun ortaya çıkmasında hangi üstbilişsel inançların rol oynadığı hakkında bilgi sahibi olmak amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya DSM-5'e gore Alkol Kullanım Bozukluğu (AKB) tanısı alan hasta grubu (n=72) ile herhangi bir psikiyatrik tanısı olmayan kontrol grubu (n=71) dahil edildi. Sosyodemografik veri formu, Alkol Kullanım Bozuklukları Tanıma Testi (AKBTT), Üstbiliş Ölçeği (ÜBÖ-30), Sürekli Öfke ve Öfke İfade Tarzı Ölçeği (SÖ-ÖİTÖ) ve Buss-Perry Agresyon Ölçeği (AÖ) kullanılmıştır.

Bulgular: AKB grubunda ÜBÖ-30 toplam, SÖ-ÖİTÖ sürekli öfke, öfke dışa ve öfke içe ve AÖ toplam puanları kontrol grubuna göre anlamlı olarak yüksek bulundu (sırasıyla $p<0,001$, $p<0,001$, $p=0,001$, $p=0,001$ ve $p<0,001$). Gruplar arasında ÜBÖ-30 ve diğer ölçeklerin korelasyon katsayıları karşılaştırıldığında, AKB grubunda sürekli öfke ve fiziksel agresyon arasında farklılık saptanmıştır (sırasıyla $z=2,035$; $p=0,042$ ve $z=2,120$; $p=0,034$). Çalışmamızda yapılan regresyon analizi sonucunda, AKB olan kişilerde agresyonu yordayan en önemli üstbilişsel inancın düşünceleri kontrol etme ihtiyacı olduğu bulunmuştur ($\beta=0.567$,$t(66)= 4.034$, $p<0.001$,$pr2 =0.20$).

Sonuç: Üstbilişsel inançlar AKB olan kişilerde yüksek oranda etkilenir ve daha fazla öfke ve agresyona neden olur. Öfkenin ortaya çıkmasında düşünceleri kontrol etme ihtiyacının önemli bir rol oynadığı gösterilmiştir.

Anahtar Kelimeler: Alkol Bağımlılığı, Üstbiliş, Öfke, Agresyon.

INTRODUCTION

Dr. Benjamin Rush defined excessive alcohol use as a disease in the 1700s and stated his treatment as abstinence from alcohol (1). With the Diagnostic and Statistical Manual of Mental Disorders Fifth, alcohol-related disorders were no longer two separate diagnostic categories as abuse and addiction, and were gathered under the heading of Alcohol Use Disorder (AUD) (2).According to World Health Organization (WHO) data, the incidence of AUD was determined as 4.1% worldwide and 7.5% in Europe in 2014 (3). It has been reported that the age of alcohol use is reduced to 12-14 years, Common ages of initation of alcohol is between the ages of 15-22, alcohol-related problems begin between the ages of 18-25, and applications for treatment are at the ages of 40 (4). At the same time, many psychiatric disorders can coexist with AUD. 37% of individuals with AUD are co-diagnosed for any other mental disorder (5). The most common comorbidities are: Another Substance Use Disorder, Mood Disorders, Anxiety Disorders, Post Traumatic Stress Disorder, and Personality Disorders, which also have higher rates of suicide (6). In addition, it has been reported that the frequency of anger, aggression and violence is higher in those with AUD (7).

Anger is expressed as an emotional state ranging from mild discomfort to violence (8). Aggression is defined as anger, anger and hateful destructive behavior with the aim of harming others physically and mentally (9). Buss and Perry analyzed aggression in four dimensions: physical aggression, verbal aggression, anger and hostility. Physical aggression and verbal aggression reflect motor behavior, including injuring and harming others. Anger, on the other hand, includes the physiological response and preparation for aggression and is related to the emotional or affective aspect of the behavior. Hostility is related to the cognitive aspect of behavior and includes feelings of ill will and injustice (10).

It is known that 50-86% of alcohol use accompanies anger and aggression attacks (11). Studies have shown that in case of aggression that occurs with alcohol use, AUD's are more prone to cognitively provocative cues (12). As cognitive processes lead from anger and aggression, researchers have suggested that metacognitive beliefs may have a role in the emergence of anger and aggression (13).

Metacognitive beliefs express the implicit or explicit knowledge that individuals have about their own cognition and the coping strategies that affect it. Metacognition, in its shortest definition, means that one can control these processes by being aware of their thinking processes (14,15). There are five sub-categories of metacognition: Positive Beliefs, Negative Beliefs (Uncontrollability and Danger), Cognitive Confidence, Need for Control of Thoughts, and Cognitive Awareness (16).

Considering the relationship between alcohol use and metacognition, the role of metacognition in AUD has now been defined intensively. It was found that beliefs abouts to the need to control thoughts predicted alcohol use (17). It is known that positive metacognitions about alcohol use are especially important in the development of problematic alcohol use and contribute independently to drink behavior (18,19). Metacognitive beliefs may play an important role in the regulation of emotion regulation skills in those with AUD (20). Again, there is a close relationship between craving behavior and metacognition in people with AUD(21).

Metacognition is known to be associated with both AUD and anger problems, but there is no study evaluating which subdomains of metacognition elicits anger in those with AUD. The aim of this study is to investigate which area of metacognition plays a role in the emergence of anger and aggression in people with AUD.

The hypotheses of this study;

1- People with AUD have different metacognitive beliefs than healthy people.

2- People with AUD have more anger and aggression than healthy people.

3. In people with AUD, there is a relationship between metacognition and anger and aggression.

4. People with AUD differ from healthy people in the mechanisms by which aggression occurs.

MATERIAL AND METHODS

Among the patients who applied to Duzce University Faculty of Medicine, Department of Psychiatry between February 2019 and January 2020, and patients who applied to Sakarya University Training and Research Hospital, Department of Psychiatry, who were diagnosed with Alcohol Use Disorder according to DSM-V diagnostic criteria after the interview were included to the study. Patients with comorbid Substance Use Disorder or alcohol withdrawal and Delirium Tremens, and patients under the influence of alcohol or any substance were excluded. A control group was formed from patients who did not have any psychiatric diagnosis. All individuals included in the study were literate, could understand and answer the given scale appropriately, and were selected on a voluntary basis. Sociodemographic Data Form, Alcohol Use Disorders Identification Test (AUDIT), Metacognition Scale-30 (MCQ-30), Trait Anger and Anger Expression Style Scale (STAXI), Buss-Perry's Aggression Questionnaire (AQ) were filled in by all participants.

The study was concluded with a total of 143 participants, 72 in the patient group and 71 in the control group. Patient selection flow is given in figure-1. In the power analysis, it is known that the mean MCQ score in healthy individuals is 70 (22) .

In this study, the required minimum sample size was calculated as 70 in each group, under 0.80 power and 0.05 Type 1 error conditions, in order to determine the statistical significance of one standard deviation difference in terms of MCQ score in patients with AUD compared to the control group, and in this direction, it is necessary to work with a total of 140 individuals has been planned (22,23).

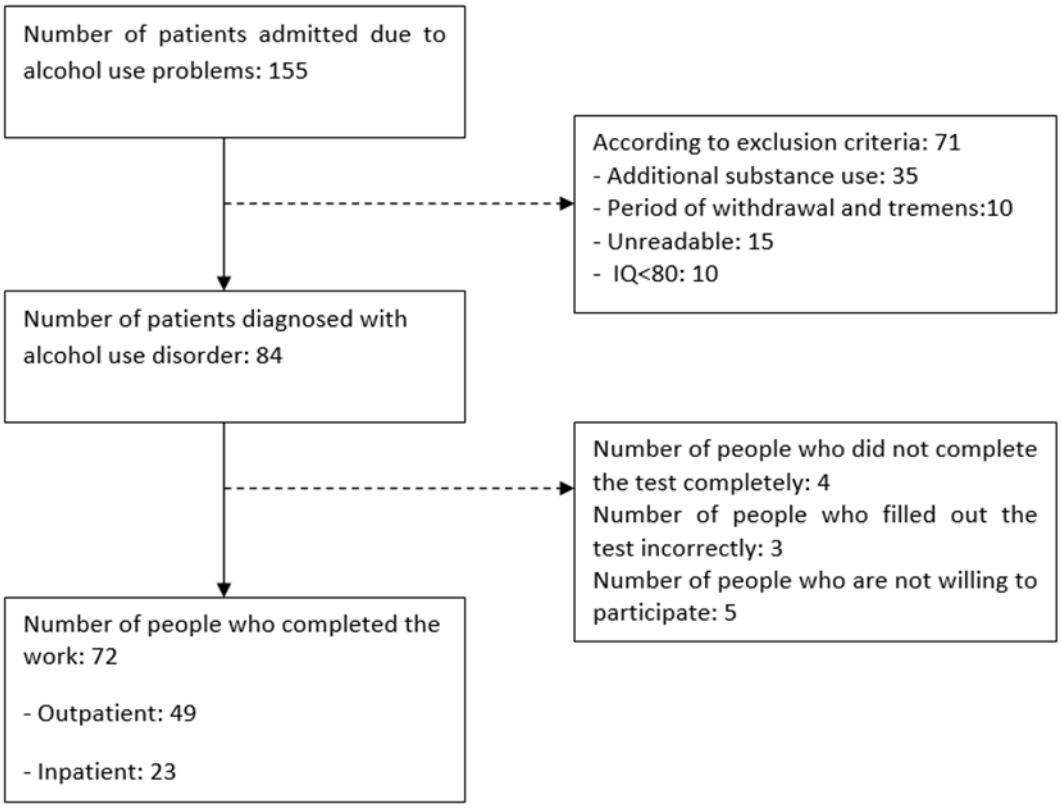

Figure 1. Flowchart for patient selection.

IRB approval for the study was proced from the Ethics Committee of Duzce University [2019/16]. All of the study procedures were in accordance with the WHO Declaration of Helsinki and local laws and regulations.

Scales

Sociodemographic Data Form: It is a form created by authors which questions participants' age, gender, marital status, education period, age at onset of alcohol use, duration of alcohol use, amount of alcohol use, additional psychiatric disease history, etc. sociodemographic characteristics.

Alcohol Use Disorders Identification Test (AUDIT): AUDIT, is developed by WHO to identify people with harmful alcohol consumption and alcohol dependence. Each question is scored on a five-point Likert scale (0-4). High scores on AUDIT were associated with alcohol dependence. Four levels of risk have been defined in WHO's AUDIT guidelines, and increased levels of risk reflect increased levels of intervention (24). The Turkish validity and reliability of AUDIT was performed by Saatçioğlu et al. in 2002 (25). The internal consistency of the scale was found to be 0.59 and 0.65 for two different interviewers.

Metacognition Questionnaire-30 (MCQ-30): The original name of the scale developed by Cartwright-Hatton and Wells is "Meta-Cognitions Questionnaire (MCQ)". Later, Wells and Cartwright-Hatton developed a short 30-item form (MCQ-30) of this scale (26). It is answered by the patient on a 4-unit Likert-type rating scale. The scores that can be obtained from the scale vary between 30 and 120, and an increase in the score indicates an increase in pathological metacognitive activity (16). The Turkish validity and reliability study of the scale was conducted by Tosun and Irak in 2008 (27). The inter-item correlation matrix of MCQ-30 is above .3, and this value indicates that the items are suitable for factor analysis. The Kaiser–Meyer–Olkin (KMO) measurement is .90 and supports the suitability of the items for factor analysis.

Trait Anger and Anger Expression Scale (STAXI): The scale developed by Spielberger, Jacobs, Russell, and Crane (1983) is a self-evaluation type scale that measures anger emotion and expression (28). The scale, which consists of 34 items and is a 4-point Likert type, includes 4 subscales: anger-in, anger-out, anger control, and trait anger. The scale evaluates trait anger and anger expression types separately. Each item is scored

between 14. Turkish adaptation was carried out by Özer (1994) (29).

Buss-Perry'sAggression Questionnaire (AQ): Developed by Buss and Durkee in 1957, the scale was revised by Buss and Perry in 1992. The aggression scale consists of 4 different sub-dimensions: physical aggression, verbal aggression, anger and hostility. Each of the questions, consisting of 29 items in total, is evaluated on a 5-point Likert type scale (10). The validity and reliability study of the AO, which was adapted into Turkish, was conducted by Evren et al. with alcohol and substance addicted patients (30).

Statistical Analysis: SPSS version 26.0 software was used for statistical analysis (SPSS Inc., Chicago IL, USA). Mean, standard deviation, number, percentage values were used for descriptive variables, and median and interquartile range values were used for data showing non-parametric distribution. Whether the numerical variables showed normal distribution or not was evaluated with the Kolmogorov-Smirnov test. Independent samples t test was used for independent groups in the comparison of two normally distributed groups, and the Mann Whitney U test was used in the comparison of the two groups in terms of normally distributed numerical variables. One-way ANOVA was used to compare more than two groups in terms of normally distributed numerical variables, and the Kruskal-Wallis test was used to compare non-normally distributed numerical variables. Correlations between normally distributed numerical variables were evaluated with Pearson correlation coefficient, and correlations between non-normally distributed numerical variables were evaluated with Spearman correlation coefficient. Regression analysis method was used to examine the relationship between the dependent variable and one or more independent variables. Statistical significance was accepted as $p < 0.05$.

RESULTS

Sociodemographic Characteristics of the Participants: The socio-demographic characteristics of the study are summarized in Table-1. While the mean age of the participants was 44.06±9.71 in the AUD group, it was 44.42±9.57 in the control group. 6 (8.3%) of 72 people in the AUD group were female, 66 (91.7%) were male, 6 (8.5%) of 71 people in the control group were female, 65 (91.5%) was male. There was no statistically significant difference between the groups in terms of age and gender (p=0.820; p=0.980, respectively). When compared in terms of marital status, the rates of being single (23.6% vs. 5.6%) and being separated (12.5% vs. 4.2%) were found to be statistically significantly higher in the AUD group compared to the control group (p=0.001). The number of siblings were significantly higher in the control group (median 4 to 3). (p=0.38)

Table 1. Comparison of the sociodemographic characteristics of the participants

		AUD (n=72)	Controls(n=71)	t,x^2	p
Age, (Mean±SD)		44.06±9.71	44.42±9.57	-.228*	0.820
Sex, n(%)	Women	6 (8.3)	6 (8.5)	.001**	0.980
	Men	66 (91.7)	65 (91.5)		
Marital status, n(%)	Married	46 (63.9)	64 (90.1)	13.889**	0.001
	Single	17 (23.6)	4 (5.6)		
	Divided	9 (12.5)	3 (4.2)		
Number of Sibling, med (min-max)		3 (1-10)	4 (1-12)	-2.091*	0.038
Smoking, n(%)		68 (94.4)	29 (40.8)	47.067**	<0.001
Substance use, n(%)		7 (9.7)	0 (0.0)	7.207**	0.013
Suicide attempt, n(%)		19 (26.4)	1 (1.4)	18.544**	<0.001
Mental disorder, n(%)		17 (23.6)	0 (0.0)	19.026**	<0.001
Psychiatric hospitalization, n(%)		27 (37.5)	0 (0.0)	32.822**	<0.001
Legal trouble, n(%)		35 (48.6)	4 (5.6)	33.290**	<0.001
Alcohol use in the family, n(%)		37 (51.4)	12 (16.9)	18.877**	<0.001
History of substance use in the family, n(%)		4 (5.6)	0 (0.0)	4.058**	0.044

*,t:independent sample t test **,x^2: chisquare test, AUD: Alcohol Use Dısorder

While the average smoking amount of 68 (94.4%) smokers in the AUD group was 36.15±18.98 packs/year, the average smoking amount of 29 smokers in the control group was 25.00±14.61 packs/year and the amount of smoking in the AUD group was found to be significantly higher than in the control group (p=0.007). Substance use in the AUD group (9.7% vs. 0.0%) was found to be significantly higher than in the control group (p=0.013).

Suicide attempt history was significantly higher in the AUD group (26.4% vs. 1.4%) compared to the control group (p<0.001).

Both psychiatric comorbid diagnosis (23.6% vs. 0.0%) and psychiatric hospitalization history (37.5% vs. 0.0%) were found to be significantly higher in the AUD group compared to the control group (p<0.001). The most common psychiatric comorbidities were Mood Disorders (n: 10), Anxiety Disorder (n: 5), Personality Disorder (n: 2).

Experiencing legal problems (48.6% vs. 5.6%) in the AUD group was significantly higher than the control group (p<0.001). Both history of familial alcohol use (51.4%; 16.9%; p<0.001) and history of family substance use (5.6%; 0.0%; p=0.044) were also significantly higher in the AUD group compared to the control group.

Comparison of AUDIT, MCQ, STAXI and AQ Scale Scores in AUD: Scale scores filled in AUD and control group are summarized in Table-2. The mean AUDIT score of the participants in the AUD group was 26.44±7.25. Positive beliefs (p=0.042), uncontrollability and danger (p<0.001), cognitive confidence (p<0.001), need to control thoughts (p<0.001) and total score (p<0.001) subscale scores of the metacognition scale in the AUD group were controlled significantly higher than the group. There was no significant difference between the groups in terms of cognitive awareness subscale scores (p=0.106). According to the scores obtained from the trait anger-anger expression style scale, trait anger (p<0.001), anger-out (p=0.001) and anger-in (p=0.001) scores were found to be significantly higher in the AUD group, while the anger control (p=0.014) scores were found in the control group.) scores were found to be significantly higher. Physical aggression (p=0.003), anger (p<0.001), hostility (p<0.001) and total (p<0.001) scores were found to be significantly higher in the AUD group compared to the scores obtained from the Buss-Perry Aggression Scale. There was no significant difference between the groups in terms of verbal aggression scores (p=0.311).

Table 2. Comparison of AUDIT, MCQ, STAXI and AQ scale scores in AUD group and control group

	AUD (n=72)	**Controls(n=71)**	**t**	**p**
	Mean±SD	**Mean±SSD**		
AUDIT	26.44±7.25	---		---
MCQ-30				
Positive Beliefs	13.88±4.90	12.28±4.38	2.050	0.042
Negative Beliefs about Uncontroll ability and Danger	15.18±4.04	12.11±3.65	4.765	<0.001
Cognitive Confidence	12.79±4.60	10.10±2.95	4.171	<0.001
Need to Control Thought	13.96±5.27	10.08±3.18	5.326	<0.001
Cognitive Self-Consciousness	15.67±4.27	14.51±4.26	1.626	0.106
Total	71.47±16.21	59.08±13.08	5.024	<0.001
STAXI				
Anger-trait	23.10±6.61	19.04±5.57	3.966	<0.001
Anger-control	20.74±5.19	22.94±5.40	-2.493	0.014
Anger-out	17.50±4.91	14.89±4.33	3.374	0.001
Anger-in	18.57±4.42	16.38±3.43	3.306	0.001
AQ				
Physical Aggression	11.58±7.66	8.27±5.33	3.009	0.003
Verbal Aggression	8.67±4.28	7.97±3.87	1.018	0.311
Anger	11.85±6.18	7.66±5.40	4.309	<0.001
Hostility	14.82±7.00	9.61±5.93	4.805	<0.001
Total	46.92±20.85	33.51±15.80	4.338	<0.001

t: independent sample t test, AUD: Alcohol Use Dısorder, AUDIT: Alcohol Use Disorders Identification Test, MCQ-30: Metacognition Questionnaire-30, STAXI: Trait Anger and Anger Expression Scale, AQ: Buss-Perry's Aggression Questionnaire

Correlations between MCQ-30, STAXI, and AQ in the AUD Group: The correlations of the scales with each other are summarized in Table-3. AUDIT score and the need to control thoughts of the MCQ-30 scale (r=0.340; p=0.003), trait anger (r=0.301; p=0.010), AQ hostility (r=0.328; p=0.005), and AQ total was observed that there was a weak positive correlation between the scores (r=0.236; p=0.046).

In the AUD group, the positive beliefs score of MCQ-30 was found to be weakly and positively correlated with STAXI anger control (r=0.289; p=0.014). In the AUD group, the uncontrollability and danger scores of the MCQ-30 were determined by the STAXI trait anger (r=0.235; p=0.047), anger-in (r=0.233; p=0.049), AQ anger (r=0.256; p=0.030) , hostility (r=0.282; p=0.016), and total score (r=0.300; p=0.010) were found to be weakly and positively correlated. Trait anger (r=0.446; p<0.001), anger-out (r=0.286; p=0.015), anger-in (r=0.413; p<0.001), anger-in of MCQ-30's cognitive confidence score in the AUD group, in STAXI scale; It was found that AQ showed weak and positive correlations with anger (r=0.308; p=0.008), hostility (r=0.384; p=0.001), total score (r=0.308; p=0.008). In the AUD group, the scores of MCQ-30's need to control thoughts were determined by the STAXI trait anger (r=0.558; p<0.001), anger-out (r=0.430; p<0.001), anger-in (r=0.476; p<0.001), AQ was moderate with physical aggression (r=0.471; p<0.001), anger (r=0.420; p<0.001), hostility (r=0.572; p<0.001), total score (r=0.553; p<0.001). In the AUD group, it was determined that the cognitive awareness score of MCQ-30 showed a weak and positive correlation with STAXI anger control (r=0.317; p=0.007).

Table 3. Examination of the correlations between MCQ-30, STAXI , and AQ in the AUD group

		MCQ-30					
	AUDIT	**Positive Beliefs**	**Negative Belief**	**Cognitive Confidence**	**Need to Control Thought**	**Cognitive Self-Consciousness**	**total**
AUDIT	1	0.112	0.046	0.097	0.340***	0.074	0.203
STAXI							
Anger-trait	0.301**	0.024	0.235*	0.446***	0.558***	0.088	0.397***
Anger-control	-0.157	0.289*	0.057	-0.089	-0.148	0.317***	0.112
Anger-out	0.088	-0.173	0.174	0.286*	0.430***	-0.145	0.174
Anger-in	0.061	-0.086	0.233*	0.413***	0.476***	-0.023	0.298**
AQ							
Physical Aggression	0.191	-0.074	0.225	0.197	0.471***	0.060	0.258*
Verbal Aggression	0.127	-0.015	0.227	0.078	0.308***	0.154	0.215
Anger	0.100	-0.068	0.256*	0.308**	0.420***	0.067	0.285*
Hostility	0.328***	0.131	0.282*	0.384***	0.572***	0.118	0.436***
Total	0.236*	-0.006	0.300**	0.308**	0.553***	0.113	0.370***

Pearson correlation test, *p<0.05;**p<0.01;***p<0.001, AUD: Alcohol Use Dısorder, AUDIT: Alcohol Use Disorders Identification Test, MCQ-30: Metacognition Questionnaire-30, STAXI: Trait Anger and Anger Expression Scale, AQ: Buss-Perry's Aggression Questionnaire

Effect of Metacognition on Aggression: Multivariate regression linear regression analysis was performed to predict the Buss-Perry's Aggression Questionnaire variable by using the subscale (Positive Beliefs, Negative Beliefs, Cognitive Confidence, Need to, Control Thought, Cognitive Self-Consciousness) variables of metacognition separately in the AUD and Control groups (table-4). As a result of the analysis, a significant regression model was obtained both in the AUD group and in the control group (F(5.66)=7.017, p<00.1;F(5.65)=2.933, p=0.02, respectively), and in the dependent variable in the AUD group. It was found that 30% of the variance (R2adjusted=0.3) was explained by the independent variables, while this rate was found to be 12% (R2adjusted=0.12) in the control group. In the AUD group, the need for control over thoughts predicted aggression positively and significantly (=0.567, t(66)= 4.034, p<0.001, pr^2=0.20). Mindfulness predicted aggression positively and significantly in the control group. (=0.,313,t(64)=2,113, p=0.038,pr^2=0.06).

Table 4. Demonstrating the effect of metacognition on aggression with multiple regression model

	Model	**Unstandardized**		**Standard**	**t**	**Sig.**	**95,0% Confidence**			
		B	**Std. Error**	**Beta**			**Lower Bound**	**Upper Bound**	**Correlation Partial**	**VIF**
AUD (n=72)	**(Constant)**	20.852	10.690		1.951	0.055	-.492	42.196		
	Positive Beliefs	-1.003	0.560	-.236	-1.789	0.078	-2.122	.116	-0.215	1.754
	Negative Beliefs	.625	0.709	.121	.881	0.382	-.792	2.041	0.108	1.906
	Cognitive Confidence	-.055	0.610	-.012	-.091	0.928	-1.272	1.162	-0.011	1.829
	Need to Control Thought	2.240	0.555	.567	4.034	<0.01	1.132	3.349	0.445	1.994
	Cognitive Self-Consciousness	-.005	0.735	-.001	-.006	0.995	-1.471	1.462	-0.001	2.283
Control (n=71)	**(Constant)**	3.524	8.825		.399	0.691	-14.101	21.149		
	Positive Beliefs	-.332	0.532	-.092	-.625	0.534	-1.395	.730	-0.077	1.728
	Negative Beliefs	-.098	0.625	-.023	-.157	0.876	-1.347	1.151	-0.019	1.663
	Cognitive Confidence	.722	0.711	.135	1.016	0.313	-.697	2.142	0.125	1.406
	Need to Control Thought	1.103	0.678	.222	1.628	0.108	-.250	2.457	0.198	1.486
	Cognitive Self-Consciousness	1.160	0.549	.313	2.113	**0.038**	0.063	2.257	0.253	1.749

DISCUSSION

AUD affects many people today and the age of alcohol use is decreasing gradually (31). People with AUD are more involved in the forensic process and experience more anger and aggression problems (7). In this study, we aimed to determine and compare metacognition in people with AUD

compared to controls and to determine the role of metacognition in the formation of aggression.

In our study, we found that metacognition in AUD differs in the sub-domains of positive beliefs, uncontrollability and danger, cognitive confidence, and the need to control thoughts. It was also found that those with AUD experienced more anger and aggression. In addition, another important result of our study is that the most important metacognitive beliefs that cause aggression in people with AUD is the need to control thoughts.

In our study, the female/male ratio of patients with AUD was found to be 1/11. Although AUD rates in women have increased recently, this rate may vary from region to country (32,33).

Moreover, smoking, additional substance use, divorce rates, additional psychiatric disorder history, suicide attempts, the rates of experiencing legal problems, and a family history of substance use were higher in the AUD group, which is consistent with the literature (34–38).

We also obtained significantly higher scores in all domains of metacognition (positive beliefs, uncontrollability and danger, cognitive confidence, and the need to control thoughts) except for cognitive awareness in people with AUD. Especially Spada suggested that metacognition plays an important role in people with AUD in his studies (39). Spada and Wells showed that alcohol use started in order to regulate negative mood in the model they proposed for AUD, and positive metacognitive beliefs about alcohol contributed to alcohol use (39). Again, negative metacognitive beliefs may play a role in alcohol use in risky situations (40). Our study was also found to be quite compatible with the literature. No difference was found in the area of cognitive awareness. This information is also compatible with the literature, and the reason why no difference was found may be that the control group also got high scores in this area (17,22).

Additionally, we found that anger and aggression were higher in the AUD group than in the control group. Being unable to cope with anger is one of the situations that can cause relapses in individuals with alcohol and substance addiction. Individuals who use alcohol and drugs can often internalize their anger or express it with an aggressive attitude. Due to the inability to control anger and not being assertive, they may be alone by experiencing deterioration in their interpersonal relationships. Loneliness and delinquency that develop because they have problems in interpersonal relationships are among the other factors that may threaten sobriety (41). In a similar study, it is observed that the trait anger level of alcohol and substance addicted patients is slightly higher than the medium level, and the mean anger-in and anger-out scores are higher than moderate (42). In our study, consistent with the literature, the fact that the trait anger, anger-out and anger-in scores were significantly higher in the AUD group compared to the control group, and the anger control scores were lower, show that the patients in this group experience anger more frequently, they experience their anger by either reflecting or suppressing it, but they have difficulty in anger control.

Aggressive behaviors and legal problems are frequently encountered in alcohol and substance addicts. Therefore, studies on aggression gain importance in this patient group. In our study, we found aggression significantly higher in the AUD group compared to the control group, while it was higher in physical aggression, anger and hostility, we did not detect any difference in verbal aggression. However, the high level of aggression in patients with AUD does not fully clarify the cause-effect relationship between addiction and aggression. It has been suggested that there may be a unidirectional relationship in the form of aggression creating a tendency for addiction or the effects of the substance used and causing aggression due to disinhibition, or a bidirectional relationship where both reinforce the other (43,44). The high level of verbal aggression in the society may have caused the absence of a significant difference.

It is seen that as the scores in the control area of metacognitive beliefs increase, AUD also increases. Again, it is seen that the need to control thoughts is positively correlated with anger and aggression. Cognitive confidence also correlates with some subscales of anger and aggression. It is seen that there is only a partial increase in the area of environmental damage of aggression as the severity of addiction increases in those with AUD. With this information, we sought an answer to the following question: Do people with AUD have metacognitive beliefs that reveal aggression?

According to multiple regression modeling, metacognition explains 30% of aggression. It was observed that the only variable that predicted aggression significantly was the belief in control of thoughts. Thought control belief alone could explain 20% of aggression. When the control group is examined, it is seen that the metacognitive belief that reveals aggression is the area of cognitive awareness. Looking at the literature, few studies have examined the relationship between metacognitive beliefs and aggression (13,45,46). We could not find a similar study in the literature on metacognitive beliefs and predicting aggression in people with AUD A longitudinal and comprehensive study on this subject also found that while metacognitive functions alone did not predict aggressive behavior, borderline and passive-aggressive personality structure and past violence history were associated. However, in this study, the sub-domains of metacognition were not evaluated separately (45). In the study examining metacognitive mental disorders and aggression, it

was thought that metacognitive functions alone do not predict aggressive behavior, but that metacognitive functions may be related to hostility arising from direct and indirect aggression (45). Salguero found that rumination predicted anger in his study of university students (46).

In people with AUD, however, there is no information about how the need to control thoughts predicts aggression. The need to control thoughts can be seen as the most important metacognitive belief of addiction-related beliefs (47). There may be several reasons for the relationship between the need for thought control and aggression in people with AUD. One of them is that people with AUD need to control their thoughts more and think that they will be punished if they cannot control them. They resort to the use of alcohol to distract from their thoughts. However, the fact that alcohol does not have this effect may cause them to drink more, not to stay away from their thoughts, and to reflect their anger towards themselves to the environment.

A second mechanism may be at the neurobiological level. It is known that serotonin plays a crucial role especially in thought control and involuntary thoughts. Serotonin mechanism is also highly impaired in people who show aggression (48,49). When this information is considered together, the irregularities related to the serotonin mechanism in people with AUD may cause the need for thought control to reveal aggression (50). Another mechanism may be via GABA, GABAergic neurons have an effect on both AUD and aggression (51,52). GABA is also involved in controlling unwanted thoughts (53). Disturbances in the GABA mechanism can also cause this situation. Therfore, it may be important for clinicians to consider drugs that act on both serotonin and GABA when working on anger management in people with AUD.

It was thought that the process of how the need to control thoughts predicts aggression in people with AUD detected in our study may be related to neurobiology and may be related to GABA and serotonin dysregulation seen in AUD, but further studies are needed to investigate this.

The limitations of our study are that it was a cross-sectional study design, additional pathologies of the patients could not be excluded, previous violence history predicting aggression and personality disorders were not evaluated. We think that its strengths are important in terms of researching which metacognitive domain reveals aggression in people with AUD and investigating the mediating role of metacognition in the emergence of aggression.

In the near future there is a need for more comprehensive studies that evaluate personality structures, violence, metacognition and psychiatric disorders that may be effective in the emergence of anger in people with AUD. In addition, it may be very interesting in studies on how interventions in different areas of metacognition change anger.

CONCLUSIONS

The use of metacognitive functions is impaired in people with AUD, there is a positive correlation between controlling thoughts in AUD and the severity of alcohol use, and cognitive therapies may be considered in the treatment. In addition, the effort to control thoughts seems to be highly responsible for aggression in people with AUD. Clinicians working on AUD and anger problems should keep in mind about the role of metacognition and should develop themselves in the field of metacognitive therapies.

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RESEARCH ARTICLE

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Anxiety and Sleep Quality in Healthcare Professionals During the Covid-19 Pandemic Process

ABSTRACT

Objective: The aim of this study is to evaluate the effects of the Covid-19 pandemic on anxiety and sleep quality in a tertiary hospital healthcare workers.

Method: The design of this study was cross-sectional and prospective and it was carried out between February- April 2021 in a tertiary hospital. By online survey method Pittsburg Sleep Quality Index (PSQI), Coronavirus Anxiety Scale (CAS) and a questionnaire created by the researchers were used for collecting the data.

Results: A total of 291 healthcare professionals, participated in the study. The mean PSQI score of patients who were detected to have coronavirus anxiety was found to be significantly higher than patients without coronavirus anxiety (p=0,005). Men's average sleep duration was found to be higher than women's (p=0,002). The mean sleep latency score was found to be higher in singles than in married people (p<0,001), and in those who did not have children (p<0,001). The sleep disorder sub-score was higher in thoose who had active night duty (p=0.028).

Conclusion: This study discovered that there is a significant relationship between sleep quality and anxiety as a result of the Covid-19 pandemic, and that healthcare workers' sleep quality suffered during this time. In order to be prepared for similar challenging conditions, health workers' physical and mental health must be protected. It is suggested that strategies for improving adverse conditions be advanced.

Keywords: Covid-19, Pandemic, Coronavirus Anxiety Scale, Pittsburg Sleep Quality Scale, Health Workers.

Covid-19 Pandemi Sürecinde Sağlık Çalışanlarında Anksiyete ve Uyku Kalitesi

ÖZET

Amaç: Bu çalışmada üçüncü basamak sağlık çalışanlarında Covid-19 pandemisinin anksiyete ve uyku kalitesi üzerine etkileri araştırılmıştır.

Gereç ve Yöntem: Araştırma prospektif ve kesitsel tipte olup, bir fakülte hastanesinde çalışanların katılımı ile Şubat-Nisan 2021 tarihleri arasında yürütülmüştür. Veriler, çevirim içi anket yöntemi ile, sosyodemografik veri değerlendirme formu, Pittsburg Uyku Kalite İndeksi (PUKİ) ve Koronavirüs Anksiyete Ölçeği (KAÖ) kullanılarak toplanmıştır.

Bulgular: Araştırmaya toplam 291 sağlık çalışanı katılmıştır. Koronavirüs anksiyetesi olduğu tespit edilen hastaların PUKİ toplam puan ortalaması, anksiyetesi olmayan hastalara göre anlamlı seviyede yüksek bulunmuştur (p=0,005). Erkeklerin ortalama uyku süresi kadınlara göre (p=0,002) anlamlı şekilde fazladır. Uyku latansı puan ortalaması ile bekâr olma (p<0,001) ve çocuk sahibi olmama durumu arasında (p<0,001) anlamlı ilişki vardır. Aktif nöbet tutmak kötü uyku kalitesi ile ilişkili bulunmuştur (p=0,028).

Sonuç: Bu çalışma, Covid-19 pandemisine bağlı anksiyetenin sağlık çalışanlarının uyku kalitelerini olumsuz yönde etkilediğini ortaya koymuştur. Tekrarlaması muhtemel zorlayıcı koşullara hazırlıklı olmak ve sağlık çalışanlarının ruh ve beden sağlığını korumak için tespit edilen risk faktörlerine yönelik iyileştirici düzenlemelere ihtiyaç vardır.

Anahtar Kelimeler: Covid-19, Pandemi, Koronavirüs Anksiyete Ölçeği, Pittsburg Uyku Kalitesi Ölçeği, Sağlık Çalışanları.

INTRODUCTION

Many variables, particularly human health, were adversely affected by the terrible effects of the Coronavirus Disease 2019 (Covid-19) pandemic. Healthcare workers have taken on a lot of responsibility during the pandemic. Prolonged and difficult working conditions have resulted in physical and mental health issues, as well as poor sleep quality, in healthcare workers who have come into direct contact with the deadly virus (1, 2).

Sleep is not a waste of time outside of daily life; rather, it is a necessity in which the body renews itself and provides the basis for a long and healthy life (3). In terms of sleep disorders, health workers, particularly doctors and nurses, were affected more than the general population during the Covid-19 pandemic. Their professional performance is likely to decrease since their health worsens as a result of anxiety and sleep disorders (4,5,6). Advance planning of therapeutic approaches for high-risk groups can significantly contribute to healthcare workers' wellbeing and readiness for difficult conditions to challenging conditions. Unlike most other studies that used general scales, a coronavirus-specific anxiety scale was used to evaluate the relationship between Covid-19 anxiety and sleep quality in healthcare workers during the Covid-19 pandemic.

MATERIAL AND METHODS

This cross-sectional, descriptive and prospective study was conducted on healthcare workers working in hospital between February-April 2021 in a tertiary hospital.

The inclusion criteria were; to be18 years age or older and to be employee at HarranUniversity Hospital. ‘’Being under the age of 18, not being an employee at Harran University Hospital, being diagnosed with a psychiatric illness such as anxiety or depression and currently using medication related to it and being diagnosed with a sleep disorder and currently using medication related to it’’were defined as exclusion criteria. A total of 319 active employees were reached. Out of 319 individuals who gave consent to participate in the study, 28 of them with regular drug use due to psychiatric illness and/or sleep disorder were excluded, and 291 participants were evaluated.

The data were obtained by asking the questions of Pittsburg Sleep Quality Index (PSQI), Coronavirus Anxiety Scale (CAS) and the sociodemographic data evaluation form created by the researchers. Participants answered the questions through the online survey method. The universe of the research consisted of doctors, nurses, health officers and other health personnel working at Harran Medical Faculty hospital. Convenience Sampling Technique was used for sample selection.

The study was started after the ethics committee approval was obtained from Harran University Clinical Research Ethics Committee on 18.01.2021.

Coronavirus Anxiety Scale (CAS): CAS was created to describe the anxiety symptoms caused by the Covid-19 pandemic process in individuals. The Turkish reliability and validity study of the scale was performed by Evren et al. (7).

There are five questions in the scale. The questions are scored in a Likert format, ranging from never (0) to almost every day (4) in the last two weeks. If the total score is less than 9, it shows that there is no anxiety about Covid-19. Dependent variables to be used in linear regression; questions asked in PSQI and CAS. The independent variables are age, gender, marital status, presence and number of children, occupation, and use of caffeine (tea, coffee) before sleep.

Pittsburg Sleep Quality Index (PSQI): This scale was developed by Buysse et al. in 1989. It evaluates sleep quality as good or bad. There are 24 questions in the scale (8). 0- 21 points can be obtained from the scale. The total score of the seven sub-components were scored between 0-3. If the total score from PSQI is less than 5, it indicates that the sleep quality is good. The Turkish reliability and validity study of the scale was performed by Ağargün et al. in 1996 and it was shown that it is a valid, standard and reliable method for measuring whether sleep quality is good or bad (9).

Data Analysis Method: Normality assumptions of the variables were examined with Skewness and Kurtosis coefficients, Kolmogorov Smirnov and Histogram tests. The Mann–Whitney test was used to compare non-normally distributed continuous variables between two groups, and the Kruskal-Wallis test was used to compare three or more groups. If a significant difference was obtained as a result of the Kruskal-Wallis test, the Mann-Whitney test with Bonferroni correction was used to determine from which groups the difference originated. In cases where the assumption of normality was met, Independent Samples T Test was used in comparisons between two groups, and one-way analysis of variance (ANOVA) was used in groups of three or more. If a significant difference was found in the ANOVA analysis, Post-Hoc analysis was applied.

IBM SPSS.23 program was used in all analyzes and $p< 0.05$ value was accepted as the level of significance.

RESULTS

A total of 291 healthcare professionals included in the study. 56.7% (n=165) of them were male and 43% (n=126) were female. The age of the participants ranged from 18 to 54. Of them 54.6% (n=159) were married and 58.4% (n=170) had no children. According to the most common occupational group distribution, 38.5% (n=112) of the participants were working as research assistants and 28.9% (n=84) as nurses and midwives.

38.8% (n=13) of the participants are those who have been working in the profession for 1-4 years. The rate of those working in internal sciences was 54.6% (n=159), and the rate of those working actively in the pandemic service was 62.5% (n=182). 70.4% (n=205) of the employees were on active duty.

The sociodemographic characteristics of the participants included in the study are shown in Table 1.

Table 1. Distribution of some sociodemographic characteristics and working status of the participants

Parameter	n	%
Gender		
male	165	56.70
female	126	43.30
Marital status		
Single/divorced	132	45.36
Married	159	54.64
Child		
yes	121	41.58
no	170	58.42
Profession		
general practitioner	11	3.78
health technician	14	4.81
research assistant	112	38.48
lecturer	17	5.84
security guard	17	5.84
cleaning staff	15	5.15
nurse/midwife	84	28.86
information technology staff	12	4.12
physiotherapist	9	3.09
Working time in the profession(year)		
<1	42	14.43
1-4	113	38.83
5-9	79	27.14
10-14	34	11.68
≥15	23	7,90
Department		
emergency	39	13.40
surgical sciences	52	17.86
internal sciences	159	54.63
Administrative/technical units	10	3.43
Intensive care	31	10.65
Active working in the pandemic service or polyclinic		
Yes	182	62.54
No	109	37.46
Previous diagnosis of sleep disorder?		
Yes	20	6.88
No	271	93.12
Active night duty		
yes	205	70.44
no	86	29.56
Number of night duty per month		
1-3	38	13.05
4-6	42	14.43
7-10	96	32.98
11-15	37	12.71
≥15	12	4.12
Consumption of caffeinated beverages (coffee, etc.) and tea 30-60 minutes before bedtime?		
yes	113	38.83
no	178	61.17

As shown in Table 2, CAS scores differ significantly according to the occupational group (p=0.045) and according to the department (p=0.003). It has been shown that the average CAS score of the personnel working in administrative and technical sciences is significantly higher than those working in surgical sciences (p=0.003). Participants who had previously been diagnosed with a sleep disorder had significantly higher CAS scores than those who had not (p=0.002).

Table 2. Comparison of coronavirus anxiety score with some sociodemographic characteristics and working status

	n	mean ± SD	median (min-max)	p
Gender				0.797*
male	165	1.85 ±3.05	1 (0 - 18)	
female	126	2.21 ± 3.5	0 (0 - 15)	
Marital status				0.891*
Single,divorced	132	2.02 ± 3.39	0 (0 - 15)	
married	159	1.99 ± 3.14	1 (0 - 18)	
Child				0.225*
yes	121	2.16 ± 3.21	1 (0 - 18)	
no	170	1.89 ± 3.28	0 (0 - 15)	
Profession				0.045**
general practitioner	11	1.55 ± 2.46	1 (0 - 8)	
health technician	14	1.5 ± 3.03	0 (0 - 11)	
research assistant	112	1.77 ± 2.81	0 (0 - 11)	
lecturer	17	0.76 ± 0.97	0 (0 - 2)	
security guard	17	2.88 ± 2.91	3 (0 - 10)	
cleaning staff	15	3.4 ± 3.78	2 (0 - 12)	
nurse/midwife	84	2.45 ± 4.23	1 (0 - 18)	
information technology staff	12	0.42 ± 0.67	0 (0 - 2)	
physiotherapist	9	2.56 ± 2.24	2 (0 - 6)	
Working time in the profession(year)				0.387**
<1	42	1.71 ± .76	0 (0 - 12)	
1-4	113	1.88 ± 3.25	0 (0 - 15)	
5-9	79	2.49 ± 3.51	1 (0 - 15)	
10-14	34	2.29 ± 3.96	0,5 (0 - 18)	
≥15	23	1.04 ± 1.4	0 (0 - 5)	
Department				0.003**
emergency	39	2.36 ± 3.44	1 (0 - 12)	
surgical sciences	52	0.81 ± 1.91	0 (0 - 10)	
internal sciences	159	2.11 ± 3.19	1 (0 - 15)	
administrative/ technical units	10	2.9 ± 3.03	2,5 (0 - 10)	
intensive care	31	2.74 ± 4.62	1 (0 - 18)	
Active working in the pandemic service or polyclinic				0.738*
yes	182	1.92 ± 3.12	0 (0 - 15)	
no	109	2.14 ± 3.47	1 (0 - 18)	
Previous diagnosis of sleep disorder?				0.002*
yes	20	4.65 ± 5.5	2 (0 - 15)	
no	271	1.81 ± 2.95	0 (0 - 18)	
Active night duty				0.402*
yes	205	2.18 ± .46	0 (0 - 18)	
no	86	1.59 ± 2.65	0,5 (0 - 11)	
Number of night duty per month				0.754**
1-3	38	2.32 ± 2.75	1.5 (0 - 10)	
4-6	42	2.07 ± 3.37	0.5 (0 - 14)	
7-10	96	1.91 ± 3.02	0 (0 - 15)	
11-15	37	2.43 ± 3.78	1 (0 - 15)	
≥15	12	3.17 ± 6.32	0 (0 - 18)	
Consumption of caffeinated beverages (coffee, etc.) and tea 30-60 minutes before bedtime				0.867**
yes	113	2.05 ± 3.49	0 (0 - 15)	
no	178	1.97 ± 3.10	1 (0 - 18)	

*Mann Whitney U test; **Kruskal-Wallis Test.

Comparison of the participants' total PSQI scores according to their sociodemographic characteristics is shown in Table 3. PSQI total scores differ according to the occupation group (p=0.002) and the working time in the profession (p=0.016). The mean score of the health technicians and research assistants is significantly higher than the security guards (p=0.014, p=0.003 respectively). The mean score of health personnel working between 5-9 years is significantly higher than those working for 15 years or more (p=0.022). The mean total score of healthcare workers with a

previous diagnosis of sleep disorder was significantly higher than those without (p=0.001). A correlation was found between the number of night duties and the total PSQI score (p=0.016). The mean PSQI total score of patients with coronavirus anxiety was significantly higher than of thoose without coronavirus anxiety (p=0.005).

Table 3. The corelation of sleep quality scores with some sociodemographic characteristics and coronavirus anxiety scores

	n	mean ± SD	Median (Min - Max.)	p
Gender				*0.130
male	165	7.26 ± 3.39	6.00 (1.00-17.00)	
female	126	6.69 ± 3.00	6.50 (1.00-17.00)	
Marital status				*0.081
Single/divorced	132	7.38 ± 3,31	7.00 (1.00-17.00)	
married	159	6.71 ± 3.15	6.00 (1.00-17.00)	
Child				*0.616
yes	121	6.90 ± 3.35	6.00 (1.00-17.00)	
no	170	7.09 ± 3.16	7.00 (1.00-17.00)	
Profession				**0.002
general practitioner	11	5.82 ± 1.94	6.00 (3.00-8.00)	
health technician	14	8.29 ± 2.79	9.00 (2.00-11.00)	
research assistant	112	7.12 ± 3.20	6.00 (1.00-17.00)	
lecturer	17	5.76 ± 2.11	5.00 (3.00-10.00)	
security guard	17	4.82 ± 1.78	5.00 (2.00-9.00)	
cleaning staff	15	6.60 ± 2.95	7.00 (1.00-11.00)	
nurse/midwife	84	7.90 ± 3.71	7.50 (2.00-17.00)	
information technology staff	12	5.75 ± 2.60	7.00 (1.00-9.00)	
physiotherapist	9	5.67 ± 2.18	6.00 (2.00-8.00)	
Working time in the profession(year)				**0.016
<1	42	6.24 ± 3.26	6.00 (1.00-15.00)	
1-4	113	7.11 ± 2.93	7.00 (2.00-17.00)	
5-9	79	7.77 ± 3.57	7.00 (3.00-17.00)	
10-14	34	6.94 ± 3.29	6.50 (1.00-14.00)	
≥15	23	5.48 ± 2.66	5.00 (1.00-13.00)	
Department				**0.391
emergency	39	6.92 ± 2.97	7.00 (1.00-13.00)	
surgical sciences	52	6.38 ± 2.71	6.00 (2.00-12.00)	
internal sciences	159	7.11 ± 3.24	6.00 (1.00-17.00)	
administrative/ technical units	10	8.40 ± 3.50	8.00 (2.00-15.00)	
Intensive care	31	7.23 ± 4.14	7.00 (2.00-17.00)	
Active working in the pandemic service or polyclinic				*0.513
yes	182	7.11 ± 3.40	6.00 (1.00-17.00)	
no	109	6.85 ± 2.93	7.00 (2.00-15.00)	
Previous diagnosis of sleep disorder?				*0.001
yes	20	10.60 ± 4.16	10.50 (3.00-17.00)	
no	271	6.75 ± 3.00	6.00 (1.00-17.00)	
Active night duty?				*0.100
yes	205	7.20 ± 3.45	6.00 (1.00-17.00)	
no	86	6.58 ± 2.62	7.00 (1.00-15.00)	
Number of night duty per month				**0.016
1-3	38	6.45 ± 3.21	6.50 (2.00-14.00)	
4-6	42	6.07 ± 2.87	5.00 (1.00-13.00)	
7-10	96	7.44 ± 3.21	7.00 (1.00-16.00)	
11-15	37	8.30 ± 3.78	8.00 (3.00-17.00)	
≥15	12	8.08 ± 3.96	8.00 (3.00-17.00)	
Consumption of caffeinated beverages (coffee, etc.) and tea 30-60 minutes before bedtime				*0.408
yes	113	7.22 ± 3.68	6.00 (2.00-17.00)	
no	178	6.88 ± 2.92	7.00 (1.00-16.00)	
coronavirus anxiety score				*0.005
no (≤9 points)	272	6.88 ± 3.17	6.00 (1.00-17.00)	
yes (≥9)	19	9.00 ± 3.48	9.00 (3.00-17.00)	

DISCUSSION

Sleep quality is an important component for physical strength and health protection. The delivery of health-care services is also a necessity that must be performed on a continuous and high-quality basis. The mental health and sleep quality of healthcare personnel should be prioritized in challenging conditions like the Covid-19 Pandemic. If this strategically important group has health issues, it is impossible to expect them to provide the necessary level of service quality. In this study 6.53% of the participants had coronavirus anxiety, and their sleep quality was worse than that of the non-anxious group.

According to a systematic review performed by Pappa et al., 23.2% of over 30,000 healthcare workers experienced anxiety during the pandemic. In their study, the rate of insomnia was reported to be 38.9% (10). The lower anxiety rates in our study could be due to participants' adaptation to pandemic conditions over time. Although our research was conducted towards the end of the pandemic sleep quality was found to be worse in those determined to have coronavirus anxiety. In China, before the pandemic, the prevalence of sleep disorders in healthcare workers was found to be 39.2% (11). Again in China, it has been shown that 78.4% of healthcare workers under the Covid-19 pandemic, had poor sleep quality and 51.7% of them experienced insomnia (12). According to the literature, sleep disorders are seen especially in female physicians and those who have long-term interactions with Covid 19 patients (13). The high prevalence of sleep disorders among healthcare professionals should be taken seriously, as sleep disorders affect not only their health but also their occupational performance.

In this study, men's average sleep duration was discovered to be significantly longer than women's. Sleep disorders were found to be more common in women during epidemic and quarantine conditions. Women's hormonal profiles, which change from puberty to the reproductive period and post-menopausal years, may play an important role in the development of sleep disorders, which can lead to psychological problems (14). However, Tu et al.'s (15) research did not find a relationship between marital status and sleep quality, married health workers had lower average sleep latency scores than single ones in our study. At the same time, it was discovered that this problem was more prevalent among those who did not have children. Badellino et al., on the other hand, discovered that living with family/partner increases the risk of psychological distress (16). Being married is also a risk factor for anxiety in anesthesiologists in the pandemic (17). Married people are generally less likely to experience mental health issues, but they might have experienced unforeseen family crises during the pandemic. Duran et al. found that single people had lower sleep quality than married people, which is consistent with our findings. Singles may have experienced quite lonely during the period of social isolation, which may have impacted their sleep quality (18).

In this study, it was discovered that those with fewer working years in the profession had significantly higher sleep latency sub-dimension scores. This can be explained by the fact that those with more professional experience have more crisis management experience. Studies showed that during the SARS epidemic among nurses, younger age was associated with mood disorder and insomnia, and higher incidence of insomnia was determined in those directly involved in the treatment or care of Covid-19 patients (19, 20). Adequate training and support can reduce the negative effect of this group's lack of knowledge and experience (21).

In this study, the occupational group had an impact on the delay in falling asleep, and health technicians' duration of falling asleep was noticeably longer than lecturers'. According to San Martin et al., during the Covid-19 pandemic, healthcare personnel who were actively involved in the diagnosis, treatment, or care of patients had experienced sleeplessness more than non-healthcare employees (22). Working in an intensive care unit and working more hours per week were linked to poor sleep quality in another study (23). Moreover, studies have shown increased anxiety and decreased sleep quality among emergency service workers during the pandemic (24, 25). It can be said that the more frequent and close contact of the employees with the patients containing deadly virüs increases their anxiety.

Zhou et al. found that the sleep quality of nurses was lower than that of doctors and medical technicians (26). This can be explained by the increased possibility of disruption in circadian rhythms due to the fact that nurses work all night with frequent night shifts (27). In a study from Italy showed that during the pandemic restrictions, the majority of participants (55.32%) reported being disturbed, and there was a strong correlation between an irregular sleep schedule and poor sleep quality. (28). The frequency of monthly night shifts was found to have a significant effect on the subjective sleep quality in our study. It was found that those who had 11–15 night duties every month had the lowest score for subjective sleep quality.

It has been reported that the increasing workload severely affects the sleep patterns of healthcare personnel, and that quality sleep requires a steady work schedule, in addition to the stress brought on by the lethal Covid-19. During the pandemic, however, medical personnel had to work nonstop to save people's lives (27). Ali Eyüpoğlu et al. found that the sleep quality of residents with fewer night duties was better (29). Ghalichi et al. also reported that shift workers had poorer sleep quality (30). In our study, like in the studies

mentioned above, it was discovered that health workers who work 11 to 15 shifts per month had poorer subjective sleep quality, longer times to fall asleep, and more dysfunction during the day. In their study examining adults' sleep quality before and during the Covid-19 pandemic, Targa et al. also discovered that participants' sleep was adversely affected by the pandemic. (31). Since our study was not a cohort study, the participants' prior sleep quality could not be assessed; however, it was found that 43.2% of the participants had poor sleep during the pandemic period.

CONCLUSION

This is one of the first studies which was performed by using CAS in Turkey. This study found that Covid- 19 is an anxiety risk factor. Anxiety related to the Covid-19 negatively impacts sleep and worsens daytime dysfunction in healthcare professionals. The Covid-19 pandemic was neither the first nor seems to be the last and new pandemics are likely to emerge. In order to be prepared for similar difficult conditions, health workers' mental health must be protected. It would be appropriate to schedule regular interviews to assess healthcare professionals' mental health and sleep quality. Personnel who are observed to have anxiety and sleep problems should be treated professionally before their clinical picture worsens.

LIMITATIONS

This study was conducted through an online questionnaire to reduce the risk of contact during the pandemic. In fact, face-to-face evaluations are always preferable, although this was not possible due to the special circumstances of the pandemic. Stronger evidence could be obtained if a multicentric study could be planned.

Situations such as ‘fear of being infected’, ‘carrying infection to home and ‘death of family members, friends and work mate due to Covid-19 ‘ should also be asked to participants, because these factors can cause negative feelings and can increase the anxiety and sleep disturbances”. It will be kept in mind for our further studies. Despite the limitations, we believe that the results of this study can contribute to future studies.

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RESEARCH ARTICLE

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Contribution to Diagnosis of Magnetic Resonance Imaging and Inflammatory Markers in Musculoskeletal Involvement of Brucellosis

ABSTRACT

Objective: Musculoskeletal involvement in brucellosis is very important. This study aimed to evaluate the magnetic resonance imaging (MRI) findings and hematological parameters as a predictive value for the diagnosis of musculoskeletal brucellosis.

Method: This prospective case-control study was conducted between June 2011 and November 2019 in a university hospital. Ninety-nine patients with the confirmed diagnosis of brucellosis without musculoskeletal involvement and forty-three brucellosis patients with musculoskeletal involvement were examined. The hematological, biochemical parameters, and radiological imaging findings of both groups were recorded. These parameters were statistically compared between the two groups.

Results: The mean age of the patients (non-involvement group) and musculoskeletal involvement groups was 44.04 ± 23.11 and 37.92 ± 24.80 years, respectively (P = 0.062). C-reactive protein (CRP) and alkaline phosphatase (ALP) levels were significantly higher in the musculoskeletal involvement group (P < 0.05). The lower lymphocyte level was statistically significant in this group. Based on the receiver operating characteristic (ROC) analysis, the sensitivity and specificity were 70% and 65% for ALP, 77% and 58% for CRP, 83% and 45% for lymphopenia, respectively. There was no statistically significant difference between the two groups in terms of the other hematological and biochemical parameters. Spondylodiscitis (34.8%) was the most common MRI finding in patients with musculoskeletal involvement.

Conclusion: Our study results show that CRP, ALP, and lymphopenia can be used as valuable markers in the preliminary diagnosis of musculoskeletal brucellosis.

Keywords: Brucellosis, Magnetic Resonance Imaging, Spondylodiscitis, Infectious Diseases.

Brusellozda Kas İskelet Tutulumunda Manyetik Rezonans Görüntüleme ve İnflamatuar Belirteçlerin Tanıya Katkısı

ÖZET

Amaç: Brusellozda kas iskelet sistemi tutulumu çok önemlidir. Bu çalışmada, manyetik rezonans görüntüleme (MRG) bulgularının ve hematolojik parametrelerin kas-iskelet sistemi brusellozu tanısında prediktif değer olarak değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Bu prospektif vaka-kontrol çalışması Haziran 2011 ile Kasım 2019 tarihleri arasında bir üniversite hastanesinde yapıldı. Kas-iskelet tutulumu olmayan bruselloz tanısı doğrulanmış 99 hasta ve kas-iskelet tutulumu olan kırk üç bruselloz hastası incelendi. Her iki grubun hematolojik, biyokimyasal parametreleri ve radyolojik görüntüleme bulguları kaydedildi. Bu parametreler istatistiksel olarak iki grup arasında karşılaştırıldı.

Bulgular: Hastaların (tutulum olmayan grup) ve kas-iskelet tutulum gruplarının ortalama yaşı sırasıyla 44.04 ± 23.11 ve 37.92 ± 24.80 yıldı (P = 0.062). C-reaktif protein (CRP) ve alkalin fosfataz (ALP) düzeyleri kas-iskelet tutulumu grubunda anlamlı olarak daha yüksekti (P < 0.05). Lenfosit miktarının düşüklüğü bu grupta istatistiksel olarak anlamlıydı. Alıcı işletim özelliği (ROC) analizine göre, duyarlılık ve özgüllük ALP için sırasıyla %70 ve %65, CRP için %77 ve %58, lenfopeni için %83 ve %45 idi. Diğer hematolojik ve biyokimyasal parametreler açısından iki grup arasında istatistiksel olarak anlamlı fark yoktu. Spondilodiskit (%34.8) kas iskelet sistemi tutulumu olan hastalarda en sık MRG bulgusuydu.

Sonuç: Çalışma sonuçlarımız, kas-iskelet sistemi brusellozunun ön tanısında CRP, ALP ve lenfopeninin değerli belirteçler olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Bruselloz, Manyetik Rezonans Görüntüleme, Spondilodiskit, Bulaşıcı Hastalıklar.

INTRODUCTION

Brucellosis is a zoonotic bacterial infection that affects numerous organs and systems and is caused by Brucella species, which are small, intracellular gram-negative coccobacillus. Although the infection can be transmitted to humans in various ways, the most common way of transmission is through the consumption of unpasteurized milk and dairy products from an infected animal. Furthermore, it can be transmitted directly through damaged skin, conjunctival instillation, and the inhalation of infectious aerosols (1, 2). The most common complication of brucellosis in humans is the infection of bones and joints. It has been reported in high-risk regions, such as the Middle East, Asia, South and Central America, and Africa. The prevalence of musculoskeletal involvement ranges from 27% in low-risk regions to 36% in high-risk areas (3).

Musculoskeletal involvement in brucellosis is often diagnosed due to pain in joints or the evidence of infection, such as pain, swelling, functional disability, heat, tenderness, and redness at any location of the musculoskeletal region (3,4). Musculoskeletal brucellosis can occur at any time and can present as sacroiliitis, peripheral arthritis, spondylitis, and osteomyelitis (3,5). In endemic regions, brucellosis must be considered in a differential diagnosis for back pain and septic arthritis. Physical examination and laboratory tests should be performed, and imaging findings should be evaluated to make a diagnosis. Direct roentgenography, computed tomography (CT), magnetic resonance imaging (MRI), and bone scintigraphy are imaging modalities for the diagnosis of musculoskeletal brucellosis (6). The early diagnosis and early treatment of complicated cases is crucial. Since human brucellosis has variable non-specific clinical manifestations, the disease tends to be overlooked. The inadequate diagnosis of brucellosis causes an increase in the rate of chronic and complicated cases. MRI findings and inflammatory markers may be useful in identifying complicated brucellosis (5). Therefore, we focused on MRI findings and inflammatory markers in patients with musculoskeletal brucellosis in this study. In patients who had MRI evidence of musculoskeletal brucellosis, CRP, ALP, and lymphocyte levels were evaluated as possible indirect inflammatory biomarkers of musculoskeletal involvement.

MATERIAL AND METHODS

Study Population: The data obtained from patients who were diagnosed with brucellosis by clinical and serological tests and referred to the Department of Radiology for detecting any musculoskeletal involvement among brucellosis outpatients and inpatients between June 2011 and November 2019 were analyzed retrospectively. The study was performed with the permission obtained from the Medical Clinical Research Ethics Committee of our university (Date:05/22/2020 Number:156). The patients' demographic data, radiological imaging and clinical findings, and laboratory results were obtained from the hospital records retrospectively. The history, laboratory and radiological data of each patient were obtained. The data of patients with multiple admissions due to brucellosis at the time of their first admission were included in the study. Patients with the etiologically confirmed diagnosis of brucellosis and whose laboratory tests were confirmed were included in the study. Furthermore, other inflammatory diseases, autoimmune and malignant diseases represented the exclusion criteria from the study. Pregnant patients and patients under 18 years of age were excluded from the study. None of the patients participating in the study received steroid therapy or took any other anti-inflammatory medication. The patients were categorized into two groups: the brucella group and the group of brucella patients with musculoskeletal involvement. Patient serums with the positive Rose Bengal test were examined by the immunocapture-agglutination technique to eliminate the factors that caused false negativity/positivity. The brucella group consisted of patients without any complications. Brucella patients with musculoskeletal involvement were evaluated as a separate group.

Diagnosis of Brucellosis: The diagnosis of brucellosis was made based on clinical and bacteriological and/or serological findings. The patient's serums were first screened by the Rose Bengal slide agglutination test (Seromed, Istanbul, Turkey). Then, the Brucella test (capt test) (Vircell SL, Granada, Spain) was performed according to the manufacturer's instructions. Antibody titers of 1/160 and above were accepted as positive for brucellosis. However, those lower than 1/160 were accepted as negative. Blood cultures were studied using the BacT/ALERT 3D (bioMérieux, France) automated blood culture system.

The isolated bacterial strains were determined by conventional methods (Gram stain, oxidase, catalase, urease tests, etc.) and a Phoenix 100 (Becton Dickinson, USA) automated system.

Diagnosis of Musculoskeletal Brucellosis: Musculoskeletal brucellosis was diagnosed by positive serological tests or positive culture with the clinical inflammatory signs of the affected regions. We retrospectively examined patients who had undergone MRI with a pre-diagnosis of musculoskeletal involvement. In our study, MRI was performed in all patients with pain and positive serological tests. Patients with musculoskeletal involvement were identified as cases of sacroiliitis, spondylitis, spondylodiscitis, paravertebral/epidural or soft tissue abscess and osteomyelitis. Musculoskeletal presentations of brucellosis were diagnosed by physical examination and compatible

laboratory findings verified by MRI features of the affected region.

Radiological Imaging: In our study, if there was a suspicion of musculoskeletal involvement (sacroiliitis, peripheral arthritis, spondylitis, spondylodiscitis, epidural or paravertebral abscess and osteomyelitis), MRI was carried out. MRI was conducted in our Radiology Department using a Siemens Magnetom Avanto Tirm+DOT System 1.5 T MRI scanner (Siemens Healthcare, Erlangen, Germany) with an appropriate coil for each location. T1 and T2-weighted without fat saturation, fat-saturated T1 and T2-weighted, STIR (Short Tau Inversion Recovery), postcontrast fat-saturated T1-weighted MRI sequences (after the administration of 15 or 20 mL of 0.5 mmol/ml gadoteric acid or 10, 15, or 20 mL of 0.5 mmol/ml gadopentetate dimeglumine) were performed on the coronal, axial, and sagittal planes. The contrast agent was given to the patients with suspected active inflammation.

Image Evaluation: In the diagnosis of spondylitis and spondylodiscitis, vertebral endplates, bodies, intervertebral discs, paravertebral soft tissue, and epidural spaces were assessed. In patients with spinal brucellosis, paraspinal, focal or diffuse involvement, epidural spreading and cord compression were evaluated. We accepted as typical MRI findings for spondilitis a hypointense signal in T1-weighted images and hyperintense signal in T2-weighted images in the vertebral corpus. For MRI diagnosis of discitis, we searched the presence of a hyperintense signal in intervertebral discs in T2-weighted images and blurring in vertebral endplates in T1-weighted images. In post-contrast T1-weighted fat-suppressed MRI of patients with spondilodiscitis, we demonstrated contrast enhancement in the vertebral endplate, intervertebral disc, and paravertebral soft tissue. In the radiological examination for the sacroiliac joint, unilateral or bilateral joint involvement, bone marrow edema, joint enlargement or narrowing, intra-articular fluid, joint irregularity (irregularity in the joint surfaces), joint sclerosis, periarticular involvement, and contrast enhancement were evaluated. In the appendicular joint involvement, bone marrow edema, joint derangement, synovial fluid, the enhancement of synovium and periarticular soft tissues were observed after the gadolinium-based contrast agent injection on MRI like as the literature data (7).

Laboratory Data: The erythrocyte sedimentation rate (ESR), complete blood count, and blood biochemistry profile were examined. Hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), lymphocyte, neutrophil and platelet count, mean platelet volume (MPV), red blood cell distribution width (RDW), C-reactive protein (CRP), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, liver enzymes, and lipid profile were recorded for each group. MPV, neutrophil/lymphocyte ratio (NLR), and platelet/lymphocyte ratio (PLR) were calculated. Blood samples were collected in tubes containing standard ethylenediaminetetraacetic acid (EDTA). All blood samples in our study were tested for hematological parameters using the same regularly calibrated analyzer (Abbott CELL-DYN 3700, United States).

Statistical Analysis: Statistical analysis was conducted using the SPSS 18.0 version program. For the evaluation of the results, standard statistical methods were employed. The average, standard deviation, minimum and maximum values of the data were revealed. Student's t-test was used to compare independent quantitative parameters with normal distribution. The Mann-Whitney U test was used to compare independent quantitative parameters without normal distribution. The chi-square test and one-way variance analysis (ANOVA) were used to compare categorical and continuous variables between the groups. The correlation between the investigated variables was determined using Pearson's coefficient linear correlation analysis. The data were evaluated at the 95% confidence interval, and $p < 0.05$ was considered significant. The receiver operating characteristic curves (ROC) analysis, area under the ROC curve (AUC), sensitivity and specificity values were evaluated.

RESULTS

The study involved 142 patients (72 females and 70 males) with a mean age of 49±17 years and diagnosed with brucellosis. There were significant differences in the mean age of patients with and without musculoskeletal involvement ($p < 0.05$). There were no significant differences in gender. There were no significant differences in the laboratory and serological findings between the groups, except for older age, high CRP and ALP levels, and lower lymphocyte level. These were found to be significant factors in predicting musculoskeletal involvement. The patients' demographic and laboratory characteristics are shown in Table 1. The distribution of brucellosis patients with musculoskeletal involvement is shown in Figure 1. MRI showed that 15 (34.8%) patients had spondylodiscitis (Figure 2), 10 (23.2%) patients had sacroiliitis (Figure 3), 8 (18.6%) patients had peripheral arthritis (Figure 4), and 10 (23.2%) patients had combined findings (soft tissue abscess and soft tissue inflammation) (Figure 5). The vertebral corpus morphology of patients with spondylodiscitis was preserved. In our study, two consecutive vertebrae were affected. And there was no cervical region involvement. In some of our cases, spondylodiscitis was accompanied by paraspinal soft tissue inflammation. The frequency of axial skeleton involvement was significantly higher than the appendicular skeleton ($p < 0.05$). In patients with musculoskeletal involvement, the mean values of CRP and ALP were significantly higher than in patients without involvement (Table 1).

Table 1. Comparison of the demographic data and laboratory values according to the presence or absence of musculoskeletal involvement in brucellosis.

Parameters	All Brucella patients n=142 Median ± IQR	Patients without musculoskeletal involvement n=99 Median ± IQR	Patients with musculoskeletal involvement n=43 Median ± IQR	P-value
Age	49.15±17.50	48.00±20.00	50.50±19.25	**0.029***
ESR	31.0338±38.00	23.0000±31.50	29.0000±38.75	0.262
CRP	26.2691±30.33	5.7000±24.56	15.2800±34.88	**0.049***
WBC	7.544±3.15	7.3000±3.20	6.4500±3.25	0.142
Neutrophil	4.7752±2.90	4.6000±2.90	4.2000±3.33	0.416
Lymphocyte	1.9979±1.10	2.1000±1.20	1.6000±0.80	**0.042***
NLR	2.9168±1.83	2.0909±1.75	2.5719±2.11	0.502
Monocyte	0.5773±0.30	0.5000±0.30	0.5000±0.30	0.960
Eosinophil	0.1397±0.20	0.1000±0.20	0.1000±0.20	0.759
Basophil	0.0348±0.10	0.0000±0.10	0.0000±0.10	0.866
Hemoglobin (Hb)	13.2979±2.35	13.5000±2.40	13.5000±2.65	0.896
RDW	14.6823±1.65	14.2000±1.70	14.0000±1.38	0.626
PC (Platelet count)	252.3972±115.50	238.0000±111.00	264.5000±134.25	0.499
MPV	8.3780±1.20	8.3000±1.10	8.1000±1.05	0.142
PLR	122.1053±81.75	120.000±77.45	135.200±93.18	0.103
BUN	15.6564±6.00	13.0000±6.50	15.0000±6.50	0.057
Creatinine	0.9545±0.28	0.8500±0.30	0.8200±0.25	0.348
HDL	41.3325±19.56	43.7300±20.46	38.1200±21.71	0.992
LDL	99.6194±45.74	87.0000±38.38	97.0000±60.38	0.204
AST	44.8087±15.00	22.0000±14.34	23.0850±14.54	0.324
ALT	38.3651±19.51	19.1700±19.45	21.5500±20.49	0.440
ALP	97.4400±39.05	83.1500±42.91	103.4500±45.36	**0.002***

* p <0.05 was considered significant.

Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell (WBC), neutrophil/lymphocyte ratio (NLR), hemoglobin (Hb), red blood cell distribution width (RDW), platelet count (PC), mean platelet volume (MPV), platelet/lymphocyte ratio (PLR), blood urea nitrogen (BUN), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)

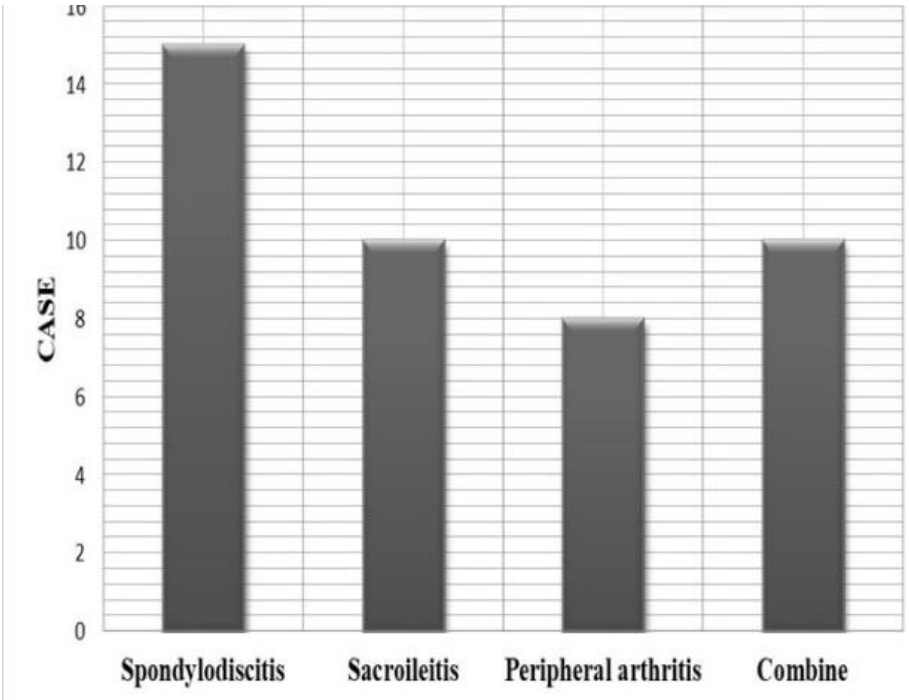

Figure 1. Distribution of brucellosis patients with musculoskeletal involvement according to clinical and radiological findings

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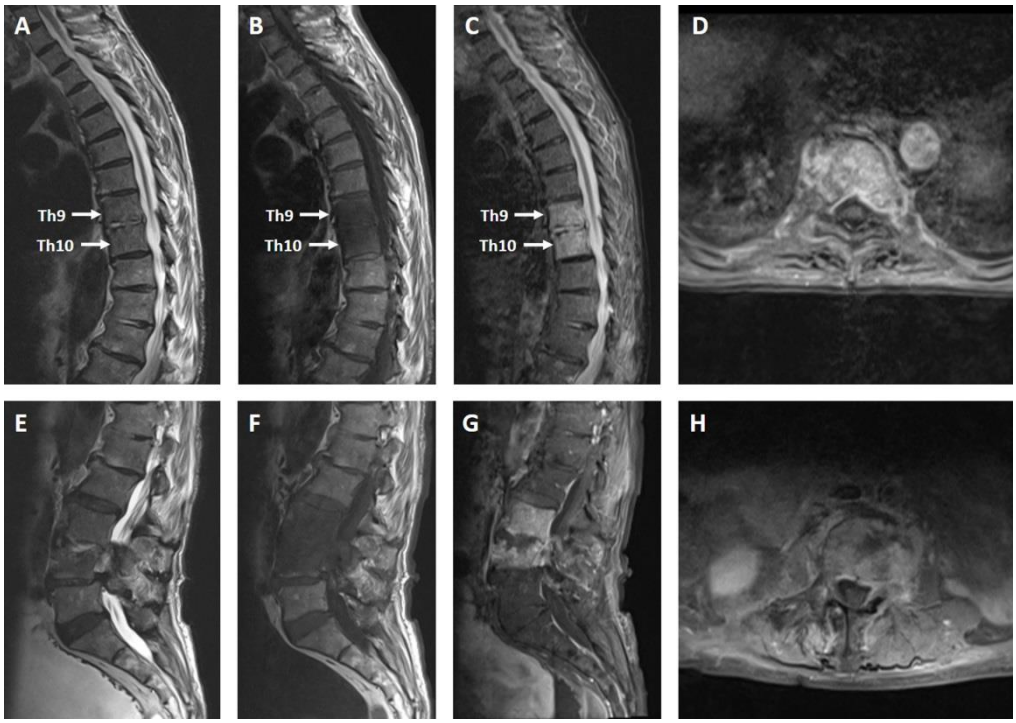

Figure 2. Thoracic spondylodiscitis: (A and B) The sagittal T2-weighted and sagittal T1-weighted images show a hypointense signals in the vertebral bodies and endplates (T9-10). (C) The sagittal STIR image shows a hyperintense signal in the vertebral bodies and endplates. (D) The contrast-enhanced T1-weighted fat-saturated axial image shows enhancement in the affected vertebra and paravertebral soft tissue. Lumbar spondylodiscitis: (E and F) The sagittal T2-weighted and sagittal T1-weighted images show a hypointense signals in the L3-L4 vertebral bodies and endplates. (G) The contrast-enhanced T1-weighted fat-saturated sagittal image shows the formation of spondylitis and the involvement of the intervertebral disc space between L3-L4 vertebral levels. (H) The contrast-enhanced T1-weighted fat-saturated axial image shows enhancement in the affected vertebra and paravertebral soft tissue.

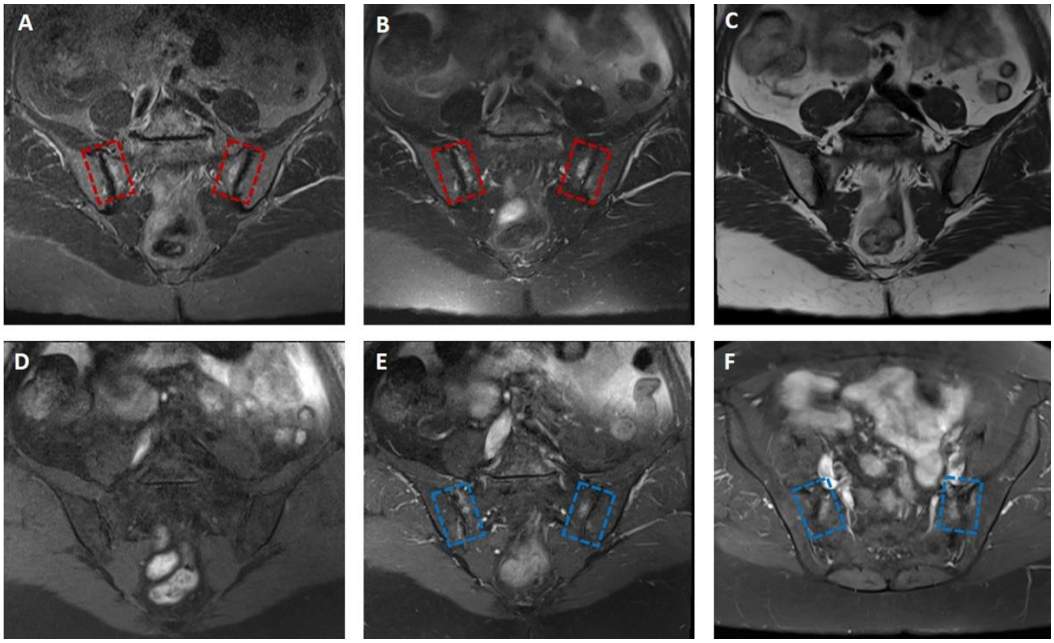

Figure 3. Bilateral sacroiliitis: (A and B) Coronal STIR (Short tau inversion recovery) and fat-saturated TSE (Turbo spin echo) T2 weighted MR sequences show bilateral hyperintense signal changes (red frames) on both iliac wings and sacral surfaces. (C and D) Coronal T1 and fat-saturated precontrast T1 weighted MR images reveal narrowing of bilateral sacroiliac joint space, irregularity on the bony faces. (E and F) Coronal and axial postcontrast fat-saturated T1 weighted MR sequences show pathological enhancements (blue frames) in subarticular bone marrow

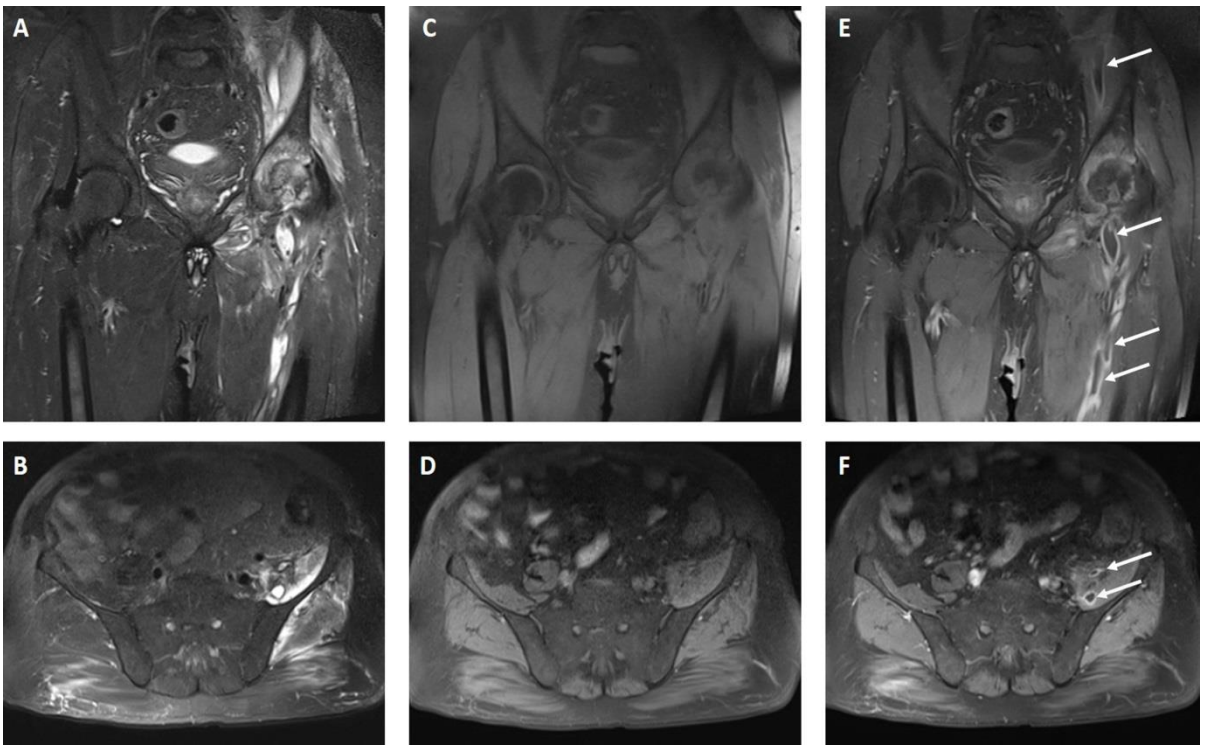

Figure 4. Left hip joint arthritis and accompanying left iliopsoas muscle abscess: (A and B) Coronal and axial fat-saturated T2 weighted MR images show diffuse hyperintense inflammatory signal changes in subarticular bone marrow of the left hip and periarticular soft tissues. (C-F) Pre- and post-contrast coronal and axial fat-saturated T1 weighted MR sequences reveal abscess formations (arrows) with peripheral enhancement in the left iliopsoas muscle and the adductor muscle planes of the left thigh.

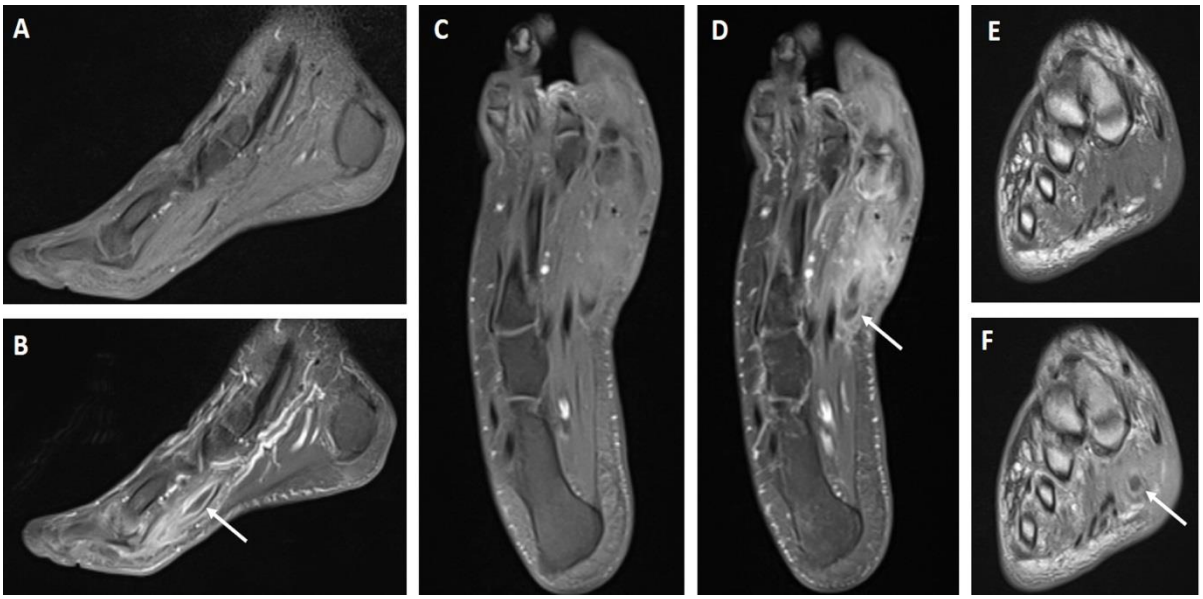

Figure 5. Right foot abscess: Pre- and post-contrast sagittal (A and B), axial (C and D), and coronal (E and F) fat-saturated T1 weighted MR images reveal inflammatory signal changes and peripherally enhancing abscess formation (arrows) on the plantar medial part of the right foot.

The frequency of lymphopenia was significantly lower in patients with musculoskeletal involvement. Among all patients, standard tube agglutination test (STA) titers ranged from 1/160 to > 1/1280. The differences in the distribution of the STA titers between patients with and without musculoskeletal involvement were statistically significant ($p = 0.001$).

The majority of the patients with musculoskeletal involvement had a higher STA titer (≥ 1/640) than patients without musculoskeletal involvement, and this difference was statistically significant ($p = 0.001$). The ROC curves and AUC value for CRP, ALP, and lymphocytes are presented in Figures 6 and 7 and Table 2.

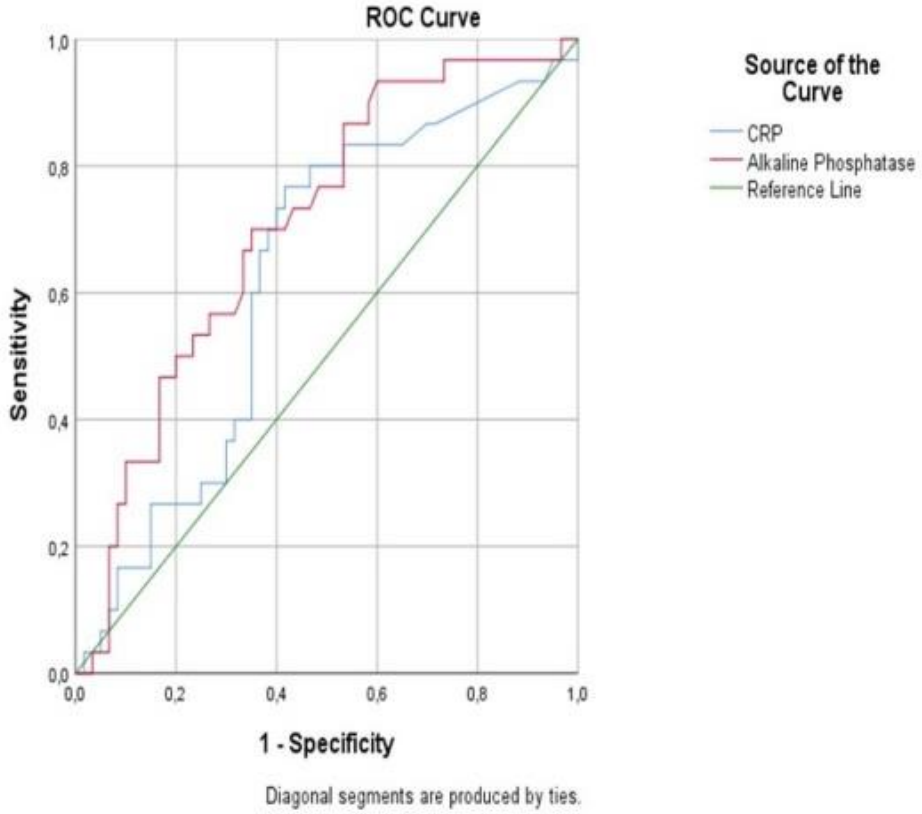

Figure 6. Receiver Operating Characteristic Curve (ROC) analysis for various cut-off levels of CRP and ALP in predicting musculoskeletal involvement in brucellosis.

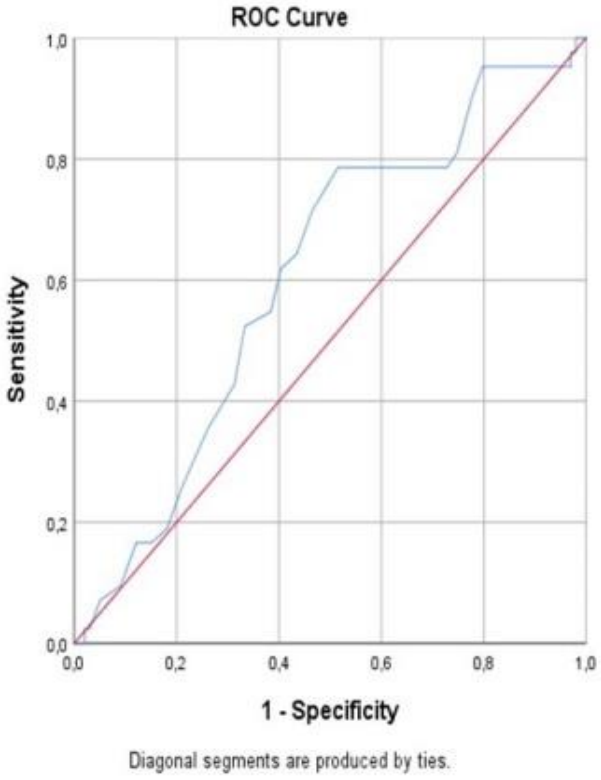

Figure 7. Receiver Operating Characteristic Curve (ROC) analysis for various cut-off levels of lymphocyte in predicting musculoskeletal involvement in brucellosis.

Table 2. The area under the curve (AUC) of CRP (C- Reaktive Protein), ALP (Alkaline phosphatase) and Lymphocytes.

Variables	AUC (95% CI)	P-Value	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
CRP	0.628 (0.505 – 0.749)	0.049	7.24	0.77	0.58	0.69	0.56	0.60
ALP	0.706 (0.595 – 0.817)	0.001	89.53	0.70	0.65	0.70	0.81	0.66
Lymphocyte	0.608 (0.509 – 0.707)	0.042	2.05	0.83	0.45	0.71	0.54	0.59

PPV: Positive predictive value
NPV: Negative Predictive Value

DISCUSSION

Brucellosis is a serious infectious public health problem in many developing countries, including Turkey, where farming is still a notable means of subsistence (2). The musculoskeletal system is commonly affected. In our study, we compared the demographic, laboratory, and imaging findings of patients with and without musculoskeletal complications to investigate the effect of easily accessible parameters in determining musculoskeletal involvement in brucellosis.

In this study, there was a significant difference between the mean ages of the patients with and without musculoskeletal involvement. Older age (50.50±19.25) was found to be a significant factor in predicting musculoskeletal involvement. While older age was found to be a risk factor for focal involvement (8), there was no significant difference between the mean ages of the patients with and without the osteoarticular affected region in some studies(9, 10).

In brucellosis, any region in the musculoskeletal system may be affected (11-13). The most important clinical presentations of musculoskeletal involvement are arthritis, spondylitis, bursitis, tenosynovitis, and osteomyelitis. Arthritis is usually observed in large joints and especially in the sacroiliac joint (14, 15). In this study, we found that spondylodiscitis was the most common site of involvement (23.2%). It was found that sacroiliitis was the most common involvement in other studies (16-18). Similar to our study, some other studies found that spondylodiscitis was more common (10, 19). There are studies indicating that peripheral arthritis is more common or spondylitis combined with sacroiliitis is more common (17, 20). Peripheral arthritis or sacroiliitis and spondylitis were more common radiological findings in some studies (16, 19, 21). As summarized in Figure 1, our results were similar to the studies.

Infectious spondylodiscitis is the involvement of anatomical structures such as the spine, intervertebral discs, paraspinal soft tissues, and epidural space by a specific organism, and it has been reported more frequently in adults over 50 years of age. Spondylodiscitis is a common and crucial musculoskeletal system complication of brucellosis infection and may cause spinal deformities and temporary or permanent neurological deficits if treatment is delayed (19, 22).

MR imaging plays a crucial role in differentiating spondylodiscitis due to brucella from other spinal pathologies such as tuberculous spondylodiscitis, pyogenic spondylodiscitis, postoperative findings in the spinal region, spinal degenerative diseases that increase with age, and vertebral metastases (23, 24). Although conventional MRI has some difficulties in differentiating acute and chronic stages of spondylodiscitis, it should be considered as the first-choice imaging method for the diagnosis, treatment, and follow-up of brucellar spondylodiscitis (25).

Vertebral metastatic processes are not affect the intervertebral disc spaces (22). Moderate epidural spread associated with intradiscal gas, varying degrees of bone sclerosis, gibbus deformity, and subligamentous extension suggest tuberculosis-related involvement rather than brucellar involvement. The lumbar segment, especially the lower lumbar region, is more involved in brucellar spondylodiscitis (26-28). Therefore, when making the differential diagnosis of vertebral involvement, the patient's history, accompanying findings, results of clinical and serological tests, and imaging features should be evaluated.

In our study, the manifesting laboratory findings in patients with osteoarticular brucellosis are high CRP, ALP, PLR, STA levels and lower lymphocyte level. Some studies reported that the level of CRP was higher in osteoarticular brucellosis than in non-osteoarticular brucellosis (29, 30). Other studies have shown that leukopenia, elevated liver enzyme level, and high CRP levels are more frequently reported findings in patients with osteoarticular involvement (31, 32). However, there are studies that do not differ significantly in terms of the frequency of leukopenia between patients with and without osteoarticular involvement. The differences in the distribution of the STA titers between these two groups were statistically significant. As the results of our study have shown, the differences in the distribution of STA titers between these patients were statistically significant in the study conducted by Ciftdogan et al (33).

MRI is a powerful tool to diagnose brucellar spondylodiscitis, especially in its early period, and paraspinal or epidural abscess, chord or root compression secondary to brucellosis (10, 18). After the gadolinium injection, signal changes in

vertebral bodies without morphologic changes and the enhancement of facet joints have been identified as specific MRI findings of brucellar spondylitis (34). Vertebral corpus morphology is almost always preserved in spinal brucellosis. Vertebral corpus integrity was preserved in our study. The involved vertebrae are generally continuous, and non-continuous vertebral involvement is rare in spondylodiscitis due to brucella (35). In our study, two consecutive vertebrae were affected. Cervical region involvement is rare in brucellar spondylodiscitis, and a few cases have been reported in the literatüre (36, 37). In our study, there was no cervical region involvement, but rather the lower lumbar region was affected.

Various researchers have demonstrated that MRI can depict soft tissue lesions in 0-89% of the cases with brucellar spondylitis (11, 19). Paravertebral soft tissue involvement was present in the majority of our patients with lumbar spondylodiscitis (Figure 2). The paraspinal soft tissue involvement is larger in size than that due to tuberculosis. The thick and irregularly enhancing abscess wall and poorly circumscribed paraspinal pathological signal are more in favor of pyogenic spondylodiscitis (38, 39). The rim-shaped, thin, and smooth enhancement of the abscess wall and the presence of a well-defined paraspinal abnormal signal in MRI are in favor of tuberculous spondylodiscitis, and narrowing of the disc distance is more frequently observed in tuberculosis-related spondylodiscitis (38-40).

Limitations of the research; The fact that it is retrospective, the number of patients is insufficient and the number of patients with and without musculoskeletal involvement due to brucellosis is not equal may cause these inflammatory markers to be insufficient in predicting the prognosis in terms of disease involvement. Another limitation is that this study focused only on MR imaging as a radiological evaluation and did not include other imaging findings (such as direct x-ray, computed tomography, and scintigraphy).

CONCLUSION

The labratory results of this study and radiological imaging findings show that older age, high CRP, ALP, STA levels and lower lymphocyte level are significant factors in estimating musculoskeletal involvement. They can be used as precious markers in the preliminary diagnosis of musculoskeletal brucellosis. We believe that these variables can be considered fast, cheap, and easily measurable new inflammatory markers in musculoskeletal brucellosis patients. More comprehensive studies are still required to investigate the predictive value of these markers in complicated brucellosis with musculoskeletal involvement.

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RESEARCH ARTICLE

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A New Predictor for Prediabetes: Chemerin

ABSTRACT

Objective: It was aimed to investigate irisin and chemerin levels in prediabetic individuals and their value in predicting prediabetes.

Method: Thirty-eight prediabetic patients aged 18-65 years (22 impaired fasting glucose (IFG), 10 impaired glucose tolerance (IGT), 6 patients with coexisting IFG and IGT) and thirty-five healthy volunteers were included in which was designed as a cross-sectional study. The basic demographic characteristics of all participants in the case and control groups were compared with the serum chemerin, irisin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein (HDL-cholesterol), low-density lipoprotein (LDL-cholesterol), triglyceride (TG), free thyroxine (sT4), and thyroid-stimulating hormone (TSH) levels.

Results: Serum chemerin level was found to be higher in the prediabetic group (p=0.03), while no significant difference was found for the irisin level between the two groups. In the multivariate logistic regression analysis, we showed that chemerin was an independent risk factor in predicting prediabetes. There was a positive correlation (p=0.01, r=0.279) between all participants' body mass index (BMI) and chemerin level and a negative correlation between irisin level and high-density lipoprotein cholesterol (HDL-C) level, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (p=0.04 r=-0.295, p=0.01 r=-0.407, respectively).

Conclusion: Chemerin is a new generation chemokine that predicts prediabetes. Studies aimed at irisin and chemerin may provide important role to prevent the prediabetes to Type 2 diabetes progression.

Keywords: Chemerin, Diabetes Mellitus, Irisin, Prediabetic State, Predictive Value of Tests.

Prediyabet İçin Yeni Bir Prediktör: Kimerin

ÖZET

Amaç: Prediyabetik bireylerde irisin ve kimerin düzeylerini ve bunların prediyabeti öngörmedeki değerini araştırmak amaçlandı.

Gereç ve Yöntem: Kesitsel bir çalışma olan dizayn edilen çalışmamıza; 18-65 yaş arası 38 prediyabetik hasta (22 bozulmuş açlık glukozu (IFG), 10 bozulmuş. glukoz toleransı (IGT), 6 IFG ve IGT birlikte bulunan hasta) ve 35 gönüllü sağlıklı kişi dahil edildi. Vaka ve kontrol grubundaki tüm katılımcıların temel demografik özellikleri ile serum kimerin, irisin, kreatinin, Alanin Aminotransferaz (ALT), Aspartat Aminotransferaz (AST), düşük dansiteli lipoprotein (LDL-kolesterol), yüksek dansiteli lipoprotein (HDL-kolesterol), trigliserit (TG), serbest tiroksin (sT4) ve tiroid stimülan hormon (TSH) düzeylerinin sonuçları karşılaştırıldı.

Bulgular: Her iki grup arasında irisin düzeyi için fark saptanmazken, kimerin düzeyi prediyabetik grupta daha yüksek saptandı (p=0.03). Yaptığımız çok değişkenli lojistik regresyon analizinde kimerinin prediyabeti ön gördürücü bağımsız bir risk faktörü olduğunu gösterdik. Ayrıca tüm katılımcıların beden kitle indeksi (BKİ) ile kimerin düzeyi arasında pozitif korelasyon (p=0.01, r=0.279); irisin düzeyi ile high density lipoprotein kolesterol (HDL-C) düzeyi ve Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) arasında negatif korelasyon (sırasıyla; p=0.04 r=-0.295, p=0.01 r=-0.407) saptadık.

Sonuç: Kimerin prediyabeti predikte eden yeni nesil bir kemokindir. İrisin ve kimerin hedefli yeni çalışmalar prediyabetin diyabete dönüşmesinin önlenmesinde önemli aşamalar sağlayabilir.

Anahtar Kelimeler: Kimerin, Diabetes Mellitus, İrisin, Prediyabetik Durum, Testlerin Prediktif Değeri.

INTRODUCTION

Type 2 Diabetes Mellitus (T2D) has a daily increasing incidence. Four hundred twenty-five million people in the adult age group around the world and approximately 7 million people in our country have T2D. It is estimated that it will increase to 700 million around the world in 2045 and that the population with T2D in Turkey will reach 12 million (1). It is important to determine patients in the prediabetic stage and to prevent progression to T2D (2).

The views on the hormonal regulation of the metabolism have changed significantly over the past two decades. It was demonstrated that some substances released from adipose tissue and muscle tissue take part in metabolic regulation and can predict T2D (3, 4).

Irisin is a cytokine secreted from many tissues such as adipose tissue, kidneys, ovaries, and stomach, especially myocytes (5). Irisin, which plays a significant role in energy consumption, insulin resistance, and thermogenesis, is a molecule with a messenger role between muscle and adipose tissue. It also plays a role on transformation of white adipose tissue to brown adipose tissue, which has a positive effect on weight control and energy balance. Known effects of irisin on the regulation of blood glucose and body weight has caused it to become a promising molecule for diabetes and obesity treatment (2, 6).

Chemerin is one of the newest members of the adipokine family that has been discovered in recent years. Mainly the liver and especially the white adipose tissue secretes it . In many studies, the role of chemerin on insulin resistance, metabolic syndrome, polycystic ovary syndrome, obesity, and T2D pathogenesis has been shown (3, 7). Chemerin, which is also an inflammatory chemokine, contributes to the proper regulation of glucose tolerance by providing insulin secretion induced glucose uptake in peripheral tissues, but its exact mechanism is not clear (8, 9). In addition, no relationship was found between prediabetes and chemerin levels in the literature.

Based on this information, it was aimed to investigate irisin and chemerin levels in prediabetic individuals and their value in predicting prediabetes in this study.

MATERIAL AND METHODS

It was designed as a cross-sectional study. Eighty individuals (n=80) aged 18-65 years who were admitted to Keçiören Training and Research Hospital (Health Sciences University), Endocrinology and Metabolic Diseases and Internal Medicine polyclinics between 01/12/2018 and 28/02/2019 and whom underwent oral 75 g glucose tolerance test (OGTT) were included in the study. Patients who have fasting blood glucose level between 100 mg/dL and 125 mg/dL were taken to the IFG group and between 140 mg/dL and 199 mg/dL were taken to the IGT group of the 2nd-hour measurement after the oral intake of 75 g of glucose. The patients with the 2nd-hour plasma glucose of ≥ 200 mg/dl in the 75 g OGTT were diagnosed with T2D (10). Seven patients were excluded from the study since they were diagnosed with T2D. The study was completed with 38 patients (n=38) diagnosed with prediabetes and 35 healthy volunteers (n=35) who were diagnosed with NGT and had no systemic disease. The exclusion criteria were being diagnosed with Type 1 or Type 2 Diabetes Mellitus (DM), pregnancy, breastfeeding, to have hematological or solid organ malignancies, chronic systemic diseases, infectious diseases, and the use of all kinds of drugs. Patients' body mass indexex (BMI), and serum creatinine, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), free thyroxine (fT4), and thyroid-stimulating hormone (TSH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) measurements, which were examined after 12-hour fasting, were recorded. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) values were calculated by the [Fasting serum glucose (mg/dl) × fasting insulin level (μU/ml)] / 405 formula. Blood analysis was done to the patient and the control groups' samples for the measurement of irisin and chemerin levels. These blood samples were centrifuged at 4000 rpm for 10 minutes, and their serums were taken to Eppendorf tubes to include 2 cc and stored at -80 ºC in a deep freezer. After patient admission was completed, serum irisin and chemerin levels were asessed using ELISA kit (USCN Life Science, Wuhan, China, with a coefficient of variation (CV) of < 10% intra-assay and < 10% inter-assay for irisin and Boster's Human/ chemerin Kit, B Valley Ave, Pleasanton, CA, USA with a CV ≤ 8% inter-assay and ≤ 5.6% intra-assay for chemerin).

Statistical Analysis: The Statistical Package for the Social Sciences version 15.0 (SPSS) was used for the analysis. The one-sample Kolmogorov-Smirnov test was used for the data distribution. Continuous variables were expressed as the median or mean SD [median (minimum-maximum)for skew-distributed, mean SD for normally distributed] whereas categorical variables as the number and percentage. For analyzing categorical variables The Fisher's exact test or a chi-square test was used and for analyzing numeric variables Student's t-test or Mann-Whitney U-test used, where appropriate. Correlation analysis was made with Spearman's rank-order or Pearson's correlation coefficient. Logistic regression analysis was made for detached predictors of prediabetes. Hosmer-Lemeshow was used for assessing model fit. P value <0.05 was accepted for statistically significance.

RESULT

In this study, 38 patients diagnosed with prediabetes (22 patients had IFG, 10 patients had IGT, and 6 patients had both) and 35 healthy individuals were included. Gender, age and BMI did not differ between groups (Table 1).

Fasting plasma glucose level, and 2nd hour plasma glucose level were higher in the prediabetes group. There was no difference between the groups in terms of ALT, AST, creatinine, total cholesterol, HDL-C, LDL-C, TG, insulin levels, and HOMA-IR measurements (p>0.05) (Table 1).

Table 1. The comparison of Demographic and laboratory findings between prediabetes and control groups

	Prediabetes (n=38)	**Control (n=35)**	**p value**
Age (year)	47 (19-60)	41 (20-65)	p>0.05
Gender (female)	28 (73.7 %)	28 (80 %)	p>0.05
BMI (kg/m²)	32 (19.5-43.5)	30.12 (19.7-44.1)	p>0.05
Creatinine (mg/dl)	0.8 (0.6-1)	0.8 (0.5-1.2)	p>0.05
ALT (IU/l)	16.5 (7-73)	19 (7-55)	p>0.05
AST (IU/dl)	19 (13-39)	18 (12-43)	p>0.05
Total Cholesterol (mg/dl)	199 (126-259)	182 (125-324)	p>0.05
HDL-C (mg/dl)	41 (33-79)	42.5 (28-58)	p>0.05
LDL-C (mg/dl)	118 (59-189)	116 (55-238)	p>0.05
Triglyceride (mg/dl)	132 (54-493)	113 (37-533)	p>0.05
Fasting plasma glucose (mg/dl)	103.5 (76-125)	91 (70-99)	**p<0.001**
Second hour glucose (mg/dl)	132.5 (65-181)	101 (77-131)	**p=0.008**
Insulin (mIU/ml)	14.4 (5.8-21)	13.9 (2.5-37.6)	p>0.05
Chemerin (ng/ml)	0.758 (0.062-14.435)	0.398 (0.039-2.284)	**p=0.03**
Irisin (ng/ml)	1.512 (0.088-2.174)	1.633 (0.093-2.451)	p>0.05
HOMA-IR	3.4 (1.69-5.07)	2.87 (0.52-10.11)	p>0.05

Chemerin levels were significantly higher in the prediabetes group [prediabetes group: 0,758 ng/ml (0,062-14,435), control group: 0,398ng/ml (0,039-2,284), p: 0.03] (Figure 1) however, irisin did not differ between groups. Also, irisin and chemerin levels did not significantly differ among IGT, IFG and IFG+IGT subgroups. Irisin and chemerin levels did not differ between obese patients (BMI≥30 kg/m²) and lean subjects (BMI<30 kg/m²). Including both patient and control groups, chemerin and BMI were significantly correlated (p=0.01, r=0.279) (Figure 2). Irisin levels were negatively correlated with the measurement of HDL-C (p=0.04, r=-0.295), and with HOMA-IR (p=0.01 r=-0.407). There was no correlation between Chemerin level and HOMA-IR level in both prediabetes and control groups. Logistic regression analysis revealed that chemerin measurements can significantly predict prediabetes (Table 2).

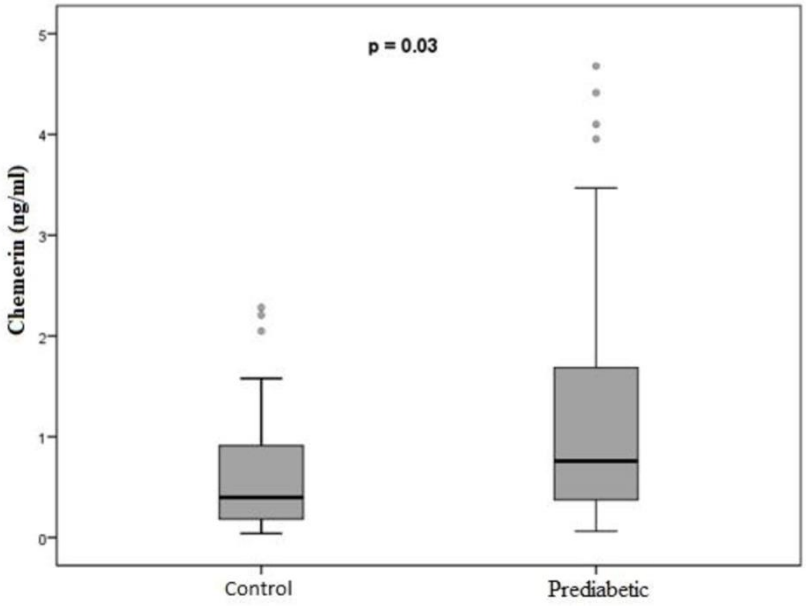

Figure 1. Comparison of the prediabetes and the control groups in terms of chemerin

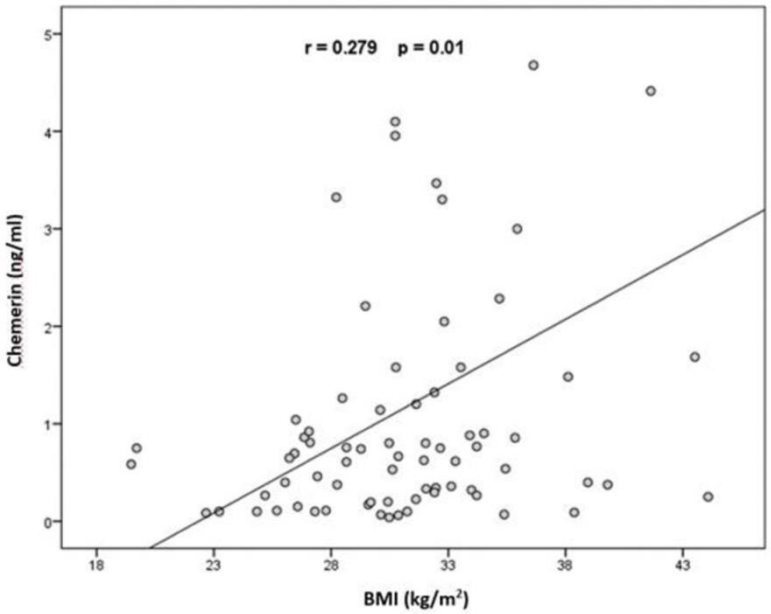

Figure 2. Correlation of the body mass index with chemerin level for all participants

Table 2. Logistic regression analysis of factor correlates with the existence of prediabetes

	OR	P	95% CI
Chemerin measurement	1.8	0.03	1.06-3.09

OR: Odds ratio; CI: Confidence Interval

-DISCUSSION

In this study, we found a correlation between prediabetes and chemerin. However, irisin levels did not differ between prediabetic patients and healthy controls. We also found that chemerin may predict prediabetes.

Since T2D has a high prevalence and can lead to severe mortality and morbidity, recent studies have focused on the early diagnosis of T2D (4, 11). Patients with T2D are usually obese or overweight. Obesity is a disease with chronic systemic inflammation. In obesity, there is an increased oxidative stress in adipocytes due to excess adipose tissue mass decreases blood circulation in adipocytes and stimulating the release of reactive oxygen species (ROS). As a result, macrophages are collected, and adipokines are released. Many inflammatory cytokines including C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-alpha), and interleukin 6 (IL-6) are released from adipose tissue. These immune phenomena are associated with obesity, with insulin resistance, and also with T2D risk (12). Chemerin is a molecule that is involved in metabolic dysregulation and is mainly secreted from adipose tissue (3). It was demonstrated that chemerin was also positively correlated with inflammatory markers in overweight individuals with T2D Chemerin level is also considered to be a good predictor for the insulin resistance level (13, 14).

In many studies on chemerin, it is remarkable that metabolic syndrome parameters are positively correlated with the chemerin level. In a study conducted by Thomas et al., it was observed that serum chemerin level was strongly correlated with metabolic syndrome parameters (15). In a study conducted on Mexican Americans, patients with T2D had higher serum chemerin levels compared to normoglycemic controls, also obese and overweight individuals had higher concentrations compared to thin controls. There was also shown a positive correlation between chemerin levels and BMI, fasting glucose, fasting serum insulin, plasma TG, and total serum cholesterol levels, and a negative correlation with HDL-C level (16). In another study conducted in a mixed ethnic group, serum chemerin levels were significantly higher in overweight and/or obese individuals, and there was a positive correlation with HOMA-IR and TG level, and a negative correlation with HDL-C level (17). In a study on the white race, a positive correlation was found between chemerin and glucose level, TG level, blood pressure (systolic and diastolic) in patients with metabolic syndrome (7). In a study involving 1431 individuals, plasma chemerin levels were higher in participants with T2D compared to non-diabetic participants. Furthermore, significantly higher chemerin levels were detected in individuals with a BMI > 30 kg/m^2 in the non-diabetic group compared to those with a BMI < 25 kg/m^2 (16). These correlations indicate that chemerin may be a new marker of metabolic syndrome and obesity and

may be a metabolic risk factor leading to insulin resistance in T2D (18). Similarly to these studies, we found a positive correlation between serum chemerin level and BMI. However, unlike this study, no correlation between chemerin level and fasting serum glucose and TG levels was found. In a recently published article, it was indicated that chemerin predicted T2D (3). In our study, it was determined that the chemerin level was correlated with BMI and was higher in the prediabetic patient group having one of the metabolic syndrome components compared to healthy individuals. As a result of the analysis in this study, it was confirmed that chemerin predicted prediabetes. In another study with patient and control groups similar to our study, no difference had been found between the groups in terms of chemerin level (19). However, we think that the limited number of participants both in our study and in that study affected the results.

It is reported that high chemerin levels are correlated with BMI (13). Although, no significant difference was found between the obese and non-obese groups' chemerin levels in our study. Actually, we observed that the chemerin level was positively correlated with BMIs of all participants. We considered that it was due to the fact that the mean BMI level was above 30 kg/m² in both groups and the number of participants with a BMI below 25 kg/m^2 was low. Although there is a consensus that chemerin is an adipocytokine that takes part in glucose homeostasis, the role of chemerin in glucose tolerance regulation still remains uncertain due to conflicting results obtained from various studies. Exogenous chemerin administration decreases serum insulin level and thus exacerbates glucose intolerance (20). It was determined that the chemerin receptor was protective against obesity but impaired insulin secretion and decreased glucose uptake in the skeleton, muscle, and adipose tissue (21). It was demonstrated that the administration of recombinant chemerin decreased insulin-stimulated glucose uptake in adipocytes under *in vitro* conditions (22).

Different results were also found in the studies on the correlation of irisin with glucose metabolism, as well as in the studies on chemerin. Serum irisin levels were demonstrated to be lower in patients with T2D compared to non-diabetic patients (23, 24). In another study, irisin was not found to be correlated with fasting c-peptide and insulin levels (25). On the other hand, irisin was found to be positively correlated with circulating insulin levels in individuals with NGT. These findings suggest that irisin take part in regulation of the beta-cell function (26). In our study, contrary to the literature, there was no significant difference in the analyses performed with the prediabetic group, control group and all participants in irisin levels. When the comparison of irisin levels with demographic data and biochemical parameters was examined, there was no significant correlation which may be due to the fact that most of the participants were obese, the number of male participants was lower, and the kit we used was different from those used in other studies. We found a positive correlation between irisin level and HOMA-IR similar to the literature (27,28). On the other hand, it has been stated in the literature that irisin level is positively correlated with insulin resistance. We think that these contradictory results are due to exercise-related and/or muscle mass-related changes in irisin level. Although the exercise-related short-term increase in irisin level varies according to age groups, the effect of exercise on the initial irisin level remains unclear (29,30). In obese individuals, lipid tissue is responsible for the basic irisin level in the blood (30). It is seen that the relationship between HDL-C and irisin levels also differs in the literature due to factors such as the difference in the ratio of white-brown lipid tissue, changes in body fat composition, etc. Elizondo-Montemayor et al. found a positive correlation between serum irisin and HDL-C levels; on the other hand, Hirsch et al. observed a negative correlation (31, 32).

Current studies have shown that irisin can accelerate the mouse beta-cells' generation especially and also increase the number of the beta-cells (33, 34). The idea of the regeneration of human pancreatic beta-cells will open a new page for the T2D management. It has been demonstrated that irisin stimulates insulin secretion due to beta-cell proliferation. The circulating levels of irisin may have positive effects on glucose tolerance and reduce insulin resistance, which can be used as an effective strategy for the treatment of T2D (35).

In a study conducted in a population consisting of healthy Korean participants, the possible relationship between serum irisin levels and T2D development in a follow-up period of approximately 2.5 years was investigated. Irisin levels were positively correlated with HbA1c and postprandial (2nd hour) glucose levels (36). When all participants were analyzed, only negative correlation of the irisin level was with the HDL-C level and HOMA-IR.

Our study has some limitations. The main limitation of our study was that it was a cross-sectional study. Furthermore, the generalizability of our results was poor due to the low number of participants. Our results represented males at a low level due to a lower number of male participants. Another limitation was that the mean BMI of our participants was high, most of our participants were overweight and obese. Furthermore, we consider that the results have been affected by the high insulin resistance in the control group. The strong aspects of our study were that the prediabetes and control groups were similar in terms of age, gender, and BMI, and participants with comorbidities were excluded from the study.

CONCLUSION

Future researches aiming chemerin could prevent the progress of prediabetes to T2D. There is a need for further studies that will reveal the effects of these cytokines more clearly and show whether they can be a part of treatment strategies in our century during which technology is developing rapidly.

Conflict of Interest: There is no conflict of interest for this project.

Ethical Approval: All procedures in this study involving human participants were in accordance with the Helsinki Declaration (1964) and its later amendments or comparable ethical standards. The ethics committee approval from Health Sciences University Keçiören Training and Research Hospital, Clinical Research Ethics Committee was obtained (Project No: 2012-KAEK-15/1780). Written and verbal informed consent was obtained from all participants.

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RESEARCH ARTICLE

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Apoptosis-associated Speck-like Protein Containing a CARD (ASC), TNF Like Factor 1a(TL-1a) and B Cell Chemoattractant Chemokine Ligand 13(CXCL-13) Expression Profiles in Familial Mediterranean Fever (FMF) Patients

ABSTRACT

Objective: This study was carried out to compare the expression levels of ASC(Apoptosis Associated Speck Like Protein Containing a CARD), TL-1a(TNF Like Factor 1a) and CXCL 13(B Cell Chemoattractant Chemokine Ligand 13) genes in FMF patients According to Tell-Hashomer Criteria and Genetic analysis result in Düzce University Research and Application Hospital with healthy controls and to determine their clinical significance in FMF.

Method: 36 patients (20 girls, 16 boys) and 12 healthy controls (7 girls, 5 boys) were included in the study. RNA was isolated from the peripheral blood of each individual and expression levels of ASC, TL-1a and CXCL 13 genes were determined. Routine biochemical parameters were also determined.

Results: CXCL 13 and TL-1a gene expression levels were significantly increased in patients with FMF, the expression level of the ASC gene was found to be increased in FMF patients, but not significantly.

Conclusion: The expression levels of these genes may be related to the pathogenesis of the disease and these genes could be used as a marker in the early diagnosis of the disease.

Keywords: FMF, ASC, CXCL13, TL-1a.

Ailesel Akdeniz Ateşi (AAA) Hastalarında Apoptosis-associated speck-like Protein Containing a CARD (ASC), TNF Like Factor 1a(TL-1a) ve B Cell Chemoattractant Chemokine Ligand 13(CXCL 13) Genlerinin Ekpresyon Düzeylerinin İncelenmesi

ÖZET

Amaç: Bu çalışma genetik analizi Düzce Üniversitesi Araştırma ve Uygulama Hastanesi'nde yapılan ve Tell-Hashomer Kriterleri'ne göre Ailesel Akdeniz Ateşi tanısı konan hastalarda ASC (Apoptosis Associated Speck Like Protein Containing a CARD), TL-1a(TNF Like Factor 1a) ve CXCL 13(B Cell Chemoatractant Chemokine Ligand 13) genlerinin ekspresyon düzeylerini sağlıklı kontroller ile karşılaştırmak ve AAA'daki klinik önemini belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışmaya 36 hasta (20 kız, 16 erkek) ve 12 sağlıklı kontrol (7 kız, 5 erkek) dahil edildi. Her bireyin periferik kanından RNA izole edildi ve ASC, TL-1a ve CXCL 13 genlerinin ekspresyon seviyeleri belirlendi. Rutin biyokimyasal parametreler de belirlendi.

Bulgular: AAA hastalarında CXCL 13 ve TL-1a gen ekspresyon seviyeleri anlamlı olarak artarken, ASC geninin ekspresyon seviyesinin arttığı ancak anlamlı olmadığı bulundu.

Sonuç: Bu genlerin ekspresyon düzeyleri hastalığın patogenezi ile ilişkili olabilir ve bu genler hastalığın erken tanısında bir belirteç olarak kullanılabilir.

Anahtar Kelimeler: AAA, ASC, CXCL 13, TL-1a.

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INTRODUCTION

FMF is a monogenic autoinfammatory disorder that is commonly observed in the Sephardic Jews (non-Ashkenaz Jews), Turks, Arabs and Armenians, and is primarily affects people of Mediterranean descent with a higher incidence. The disease is characterized by recurrent fever and recurrent peritonitis, pleuritis, arthritis or erysipelas-like rash (1).

In 1997, the International FMF Consortium and the French FMF Consortium identified the FMF gene using the positional cloning method simultaneously and independently of each other. This gene was called the MEFV gene, which consists of the initials of the Mediterranean FeVer, which means “Mediterranean fever, inspired by the region and symptoms of the disease. The MEFV gene is localized at 16p13.3 in the short arm of chromosome 16 and it has been reported that the 3.7 kilobase length gene consisting of 10 exons is encoding a transcription “marenostrine / pyrin which performs a protein synthesis of 781 amino acids (2).

The ASC(Apoptosis Associated Speck Like Protein Containing a CARD) gene is a cytosolic protein that encodes the binding protein consisting of two protein-protein interaction domains, the C-terminal caspase region (CARD) and the N-terminal pyrin region (PyD). It plays a key role in apoptosis and inflammation. In cell type-specific apoptosis, it supports caspase-mediated apoptosis including caspase-8 and caspase-9 (3).

The protein encoded by TL-1a is a cytokine of the tumor necrosis factor (TNF) ligand family. TNFRSF25 and TNFRSF6B are receptors for TL-1a. It acts as an autocrine factor to induce apoptosis in endothelial cells. It has been found that this cytokine also inhibits endothelial cell proliferation and can therefore function as an angiogenesis inhibitor. This gene supports Caspase activation (4).

CXCL-13(B Cell Chemoattractant Chemokine Ligand 13) is an antimicrobial peptide and chemokine that are strongly present in the follicles of the spleen, lymph nodes and Peyer's plaques. When B lymphocytes are confronted with T cells and macrophages, they provide migration by increasing calcium intake. It also allows B lymphocytes to return to follicles. CXCL-13 is chemotactic for B lenphocytes (5).

In addition to whole exome sequencing to reveal the roles of genes associated with different diseases in the etiopathogenesis (6-24), the detection of the expression levels of the genes also important for exactly understanding of their function and role to maintaining cellular homeostasis and viability (25,26). This study was conducted to determine the expression levels of ASC, TL-1a and CXCL-13 genes, demographic characteristics and to determine whether there is any relationship between expression levels of those genes and all of hemogram, sedimentation rate, CRP (C-reactive protein), Serum Amyloid A levels from patients and control group who applied to Duzce University Research and Application Hospital Pediatric Clinic with the diagnosis of FMF.

MATERIAL AND METHODS

The study included 36 patients with FMF and a control group of 12 subjects. The age, sex, weight, length, duration of the disease (years), kinship between the parents and family history of FMF disease were questioned. Patient duration, symptoms (abdominal pain, chest pain, joint pain, erythema, fever), diagnosis and colchicine use for many years, response to treatment, history of chronic kidney disease (history of amyloidosis), history of appendectomy, MEFV gene sequence analysis results were recorded. Routine tests such as leukocyte count, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), Serum Amyloid A biochemical parameters were examined.

For the determination of ASC, TL-1a, CXCL-13 gene expression levels, 2 cc blood was taken from each individual into EDTA tube and RNA was isolated using PureLink® RNA Mini Kit. cDNA was synthesized from the isolated RNA using RT First Strand kit (Qiagen). The mRNA levels of the target genes (ASC, CXCL-13, TL-1a) of the patient group with FMF and the control group were determined by Real Time PCR. For the Real-Time PCR reaction, the TaqMan® Gene Expression Kit from Thermo Fisher Scientific Inc USA was used and the reactions were performed on the Applied Biosystems 7500 Fast Sequenced Detector (PE Applied Bio-systems, Foster City, CA) via Real-Time PCR. The GAPDH was used as the internal control. Expression levels of ASC, TL-1a, CXCL-13 genes were detected by quantitative real time polymerase chain reaction (qRT-PCR) method and standardized by expression of GAPDH gene.

Statistical analysis

SPSS 17.0 (SPSS, Inc., Chicago, Illinois, USA) statistical program was used to evaluate the data of the current study. Mann-Whitney U Test was used for paired comparisons. Additionally Kruskal-Wallis Test was used for more than two comparisons. Correlation test was used to investigate the relationship between two groups. The relationship between two categorical (noun or graded) variables were detected via the cross-tab analysis. Results are given as mean ± standard deviation. The statistical significance level (p value) was accepted as 0.05 in all tests.

RESULTS

The distributions of the MEFV Gene Variant in Patient with FMF were given in the table 1 (Table 1). There was no significant difference in age and sex between patients with FMF and healthy controls ($p = 0.13$ and $p > 0.05$, respectively) (Table

2). The mean disease duration was calculated as 4.9 years. Leukocyte count, Sedimentation rate, CRP and Serum Amyloid A levels were not significantly different between patients and healthy controls (p = 0.13, p = 0.14, p = 0.37 and p = 0.39, respectively) (Table 2).

Although there was no significant difference between patients with FMF and control groups in terms of ASC gene expression levels, this gene expression was higher in FMF patients than in the control group (p> 0.05) (Table 2) (Figure 1).

The expression levels of TL-1a and CXCL-13 were found to be significantly higher in the FMF patient group than in the control group (p = 0.02 and p = 0.03, respectively) (Table 3) (Figure 2,3). A significant correlation was found between ASC expression level and CXCL-13 expression level and between CXCL-13 and TL-1a expression level (p = 0.05 and p = 0.000). There was no significant relationship between ASC and TL-1a expression level (p = 0.48) (Table 4) (Figure 4,5,6)

When the relationship between the expression levels of genes and complaints of the cases to be taken into consideration; The relationship between CXCL-13 and fever (p = 0.00; r: 0.45), the relationship between CXCL-13 and abdominal pain (p = 0.01; r: 0.35), the relationship between CXCL-13 and mutation (p = 0.04; r: 0.29), the relationship between CXCL-13 and TL-1a (p = 0,000; r = 0.540), the relationship between TL-1a and fever (p = 0.01; r = 0 , 35), the relationship between TL-1a and abdominal pain (p = 0.01; r = 0.34), the relationship between TL-1a and symptom year (p = 0.01; r = 0.35) were statistically significant (Table 5).

Table 1. Distrubitions of *MEFV* Gene Variant in Patient with FMF

FMF Gene variation Tıpe	**Zygosity**	**Patient n (%)**
E148Q	Homozygous	6(16.6)
R202Q	Homozygous	9 (25)
M680I	Homozygous	3 (8.3)
M694V	Homozygous	9 (25)
V726A	Homozygous	1 (2.7)
M694V and R202Q	Homozygous / Homozygous	2 (5.6)
M694V and M680I	Homozygous / Homozygous	2 (5.6)
M694V and E148Q	Heterozygous / Heterozygous	2 (5.6)
E148Q and M680I	Heterozygous / Heterozygous	2 (5.6)

n_Number of patients
FMF: Familial Mediterranean Fever

Table 2. Demographic, clinical and laboratory characteristics of FMF patients and controls

Parameters	Control average±SD	Patient average±SD	P Value	Z Value
Age(M±SD)(min-max)	8.8±4(3-15)	10.8±4(3-18)	0.13	-1.50
Genders	7 (%58.4)5 (%41,6)	20 (%55.5)16 (%44.5)	0.74	-0.33
Height (cm)	132.4±21.9(109-165)	139.3±22.0(85.1-168.8)	0.3	-1.03
Weight (kg)	33.383±18.102(15-68.70)	36.894±15.282 (12,70-65,40)	0.43	-0.78
Fever (+)	1	18	**0.01**	-2.52
Abdominal pain (+)	2	28	**0.00**	-3.74
Joint pain (+)	1	24	**0.00**	-3.46
Erythema (+)	0	2	0.41	-0.82
Chest pain (+)	1	12	**0.09**	-1.67
Symptom year	-	4.9±4(0-15)	**0.00**	-4.30
Colchicine year	-	1.8±2(0-10)	**0.01**	-2.76
Colchicine response (+)	0	19	**0.01**	-2.67
Cr. Renal Failure (+)	0	2	0.41	-0.82
Appendectomy(+)	0	1	0.56	-0.57
Parent kinship(+)	2	2	0.42	-0.81
Family history(+)	2	23	**0.01**	-2.46
Mutation(+)	0	36	0.00	-5.24
CRP(0-0,5 mg/dl)	0.2±0.2 (0,01-0,93)	0.3±0.9 (0,01-3,83)	0.38	-0.88
Sedimentation(0-20) mm/h)	9.7±10.9 (2-40)	13.3±11.7 (1-45)	0.15	-1.44
SAA(1000-5000ng/ml)	3540.7±2425.7 (283-8314)	3302.9±3532.7 (208-10940)	0.39	-0.85
Leukocyte count	9.4±2.6 (5,40-13,80)	8.1±2.5 (5,20-17,10)	0.13	-1.51
ASC(FoldChange)($2^{-\Delta CT}$)	5.50±3.65 (1,74-13,17)	6.12±4.32 (1,03-15,45)	0.79	-0.26
CXCL13(FoldChange) ($2^{-\Delta CT}$)	0.67±0.67 (0,16-2,66)	2.37±2.50 (0,03-9,99)	**0.03**	-2.09
TL1a(FoldChange) ($2^{-\Delta CT}$)	0.41±0.23 (0,07-0,87)	1.28±1.09 (0,03-3,48)	**0.02**	-2.26

mean±standart deviation:(M±SD) minimummaximum: min-max

Table 3. Comparison of ASC, CXCL-13 and TL-1a Expression Levels in Control and FMF Patient Groups

Parameters	Control average±SD (min-max)	Patient average±SD (min-max)	P	Z
ASC(FoldChange)($2^{-\Delta\Delta C}{}_t$)	5.50±3.66 (1.74-13.17)	6.12±4.33(1.03-15.45)	0.79	-0.26
CXCL-13(FoldChange) ($2^{-\Delta\Delta C}{}_t$)	0.678±0.67 (0.16-2.66)	2.38±2.50 (0.03-9.99)	**0.03**	-2.09
TL1a(FoldChange) ($2^{-\Delta\Delta C}{}_t$)	0.41±0.23 (0.07-0.87)	1.28±1.09 (0.03-3.48)	**0.02**	2.26

mean±standart deviation:(M±SD) minimum-maximum: min-max

Table 4. Association of ASC, CXCL-13 and TL-1a Expression Levels with Other Parameters in FMF Patient Group

Parameters	ASC		CXCL-13		TL-1a	
	P	r	P	r	P	r
Age	0.86	0.30	0.86	0.30	0.53	0.11
Height	0.78	0.49	0.54	0.10	0.53	0.11
Weight	0.93	-0.01	0.83	0.04	0.85	0.03
Fever	0.52	0.11	**0.03**	0.36	0.11	0.27
Abdominal pain	0.94	0.01	0.28	0.19	0.12	0.26
Joint pain	0.50	-0.12	1.00	0.00	1.00	0.00
Erythema	0.34	-0.16	0.89	0.02	0.95	-0.01
Chest pain	0.87	-0.03	0.38	0.15	0.79	0.04
Symptom year	0.45	-0.13	0.88	-0.03	0.31	0.17
Colchicine year	0.48	-0.12	0.96	-0.01	0.87	-0.03
Colchicine response	0.60	-0.09	0.89	-0.02	0.94	0.01
Cr. Renal Disease	0.45	0.13	0.64	-0.08	0.95	-0.01
Appendectomy	0.17	-0.24	0.32	0.17	0.23	-0.20
Parent Kinship	0.45	0.13	0.27	-0.19	0.26	0.19
Family History	0.43	0.14	1.00	0.00	0.94	-0.01
Mutation	0.64	-0.08	0.57	0.10	0.46	-0.13
CRP	0.23	-0.20	0.99	-0.00	0.88	0.03
Sedimentation	0.12	-0.26	0.39	0.15	0.33	0.17
SAA	0.29	-0.18	0.66	-0.07	0.24	0.20
Leukocyte count	0.11	-0.27	0.95	0.01	0.48	0.12
ASC	-	1.00	**0.05**	0.32	0.48	0.12
CXCL-13	**0.05**	0.32	-	1.00	**0.00**	0.50
TL1-a	0.48	0.12	**0,00**	0,50	-	1.00

Table 5. The Relationship Between ASC. CXCL-13 and TL-1a Expression Levels and Other Parameters

Parameters	ASC		CXCL-13		TL-1a	
	P	r	P	r	P	r
Age	0.79	-0.04	0.56	0.09	0.15	0.21
Height	0.81	-0.04	0.37	0.13	0.17	0.20
Weight	0.54	-0.09	0.58	0.08	0.25	0.17
Fever	0.66	0.06	**0.00**	0.45	**0.01**	0.35
Abdominal pain	0.66	-0.06	**0.01**	0.35	**0.01**	0.34
Joint pain	0.54	-0.09	0.21	0.18	0.31	0.14
Erythema	0.36	-0.13	0.73	0.6	0.64	-0.012
Chest pain	0.71	-0.06	0.11	0.24	0.57	0.08
Symptom year	0.57	-0.08	0.11	0.23	**0.01**	0.35
Colchicine year	0.46	-0.10	0.38	0.12	0.60	0.07
Colchicine response	0.55	-0.09	0.39	0.12	0.53	0.09
Cr. Renal diseaese	0.41	0.12	0.76	-0.04	1.00	0.00
Appendectomy	0.14	-0.21	0.23	0.17	0.23	-0.17
Parent Kinship	0.80	0.03	0.26	-0.16	0.24	0.17
Family History	0.81	0.03	0.11	0.23	0.42	0.11
Mutation	0.91	-0.01	**0.04**	0.29	0.17	0.20
CRP	0.20	-0.18	0.53	-0.09	0.95	0.00
Sedimentation	0.17	-0.20	0.29	0.15	0.26	0.16
SAA	0.29	-0.15	0.49	-0.10	0.61	0.07
Leukocyte Count	0.37	-0.13	0.63	-0.07	0.88	-0.02
ASC	-	1.00	0.11	0.23	0.29	0.16
CXCL13	0.11	0.23	-	1.00	**0.00**	0.54
TL1a	0.29	0.16	**0.00**	0.54	-	1.00

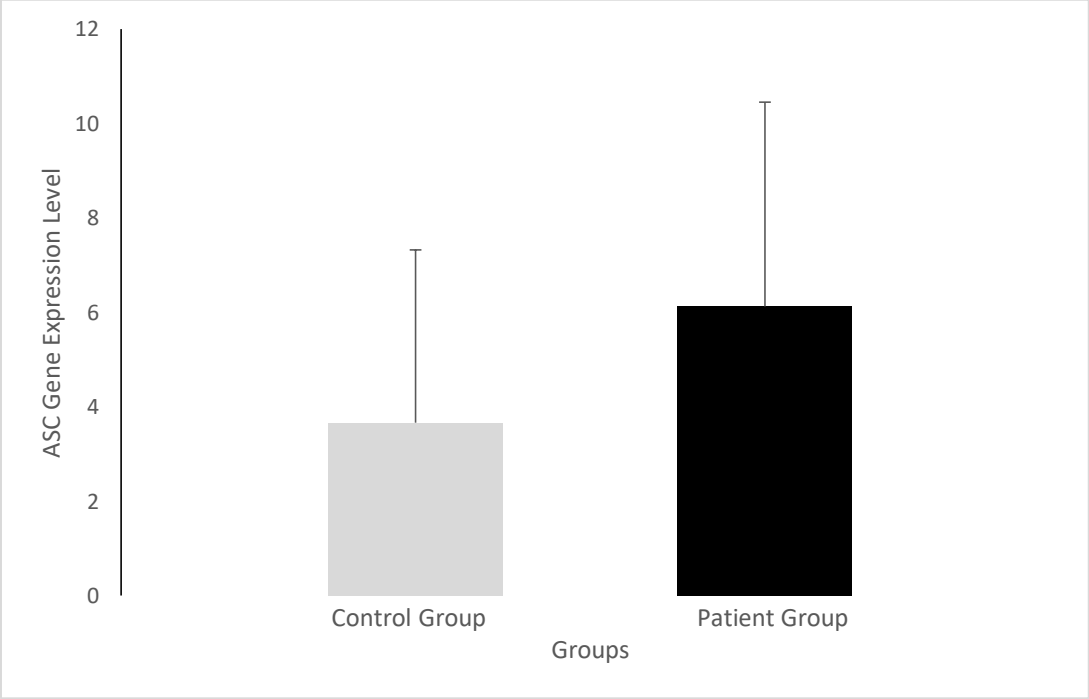

Figure 1. Comparison of ASC expression levels in patients and control groups

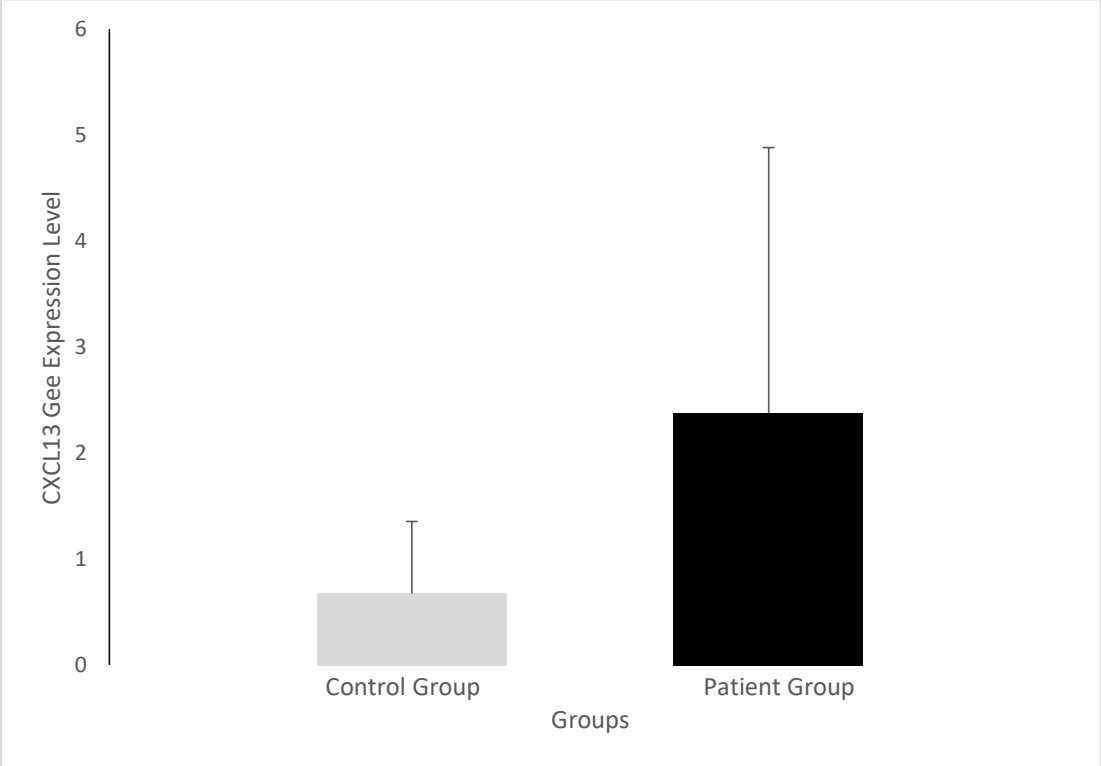

Figure 2. Comparison of CXCL-13 expression levels in patients and control groups

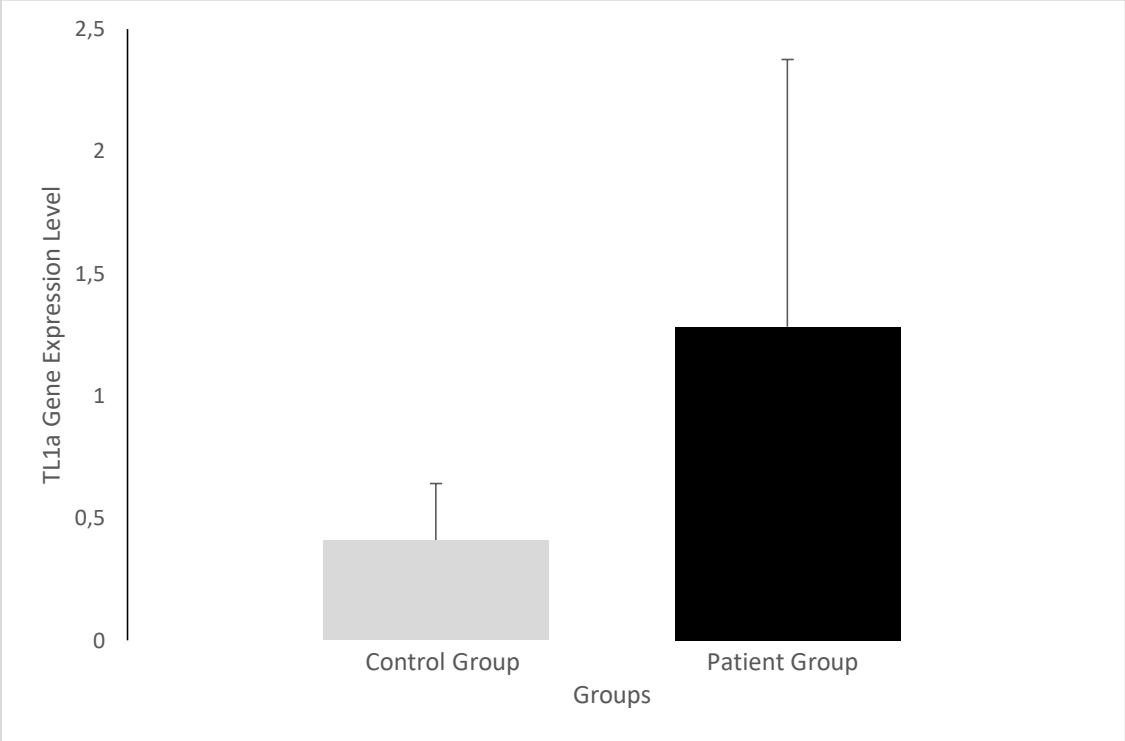

Figure 3. Comparison of TL-1a expression levels in patients and control groups

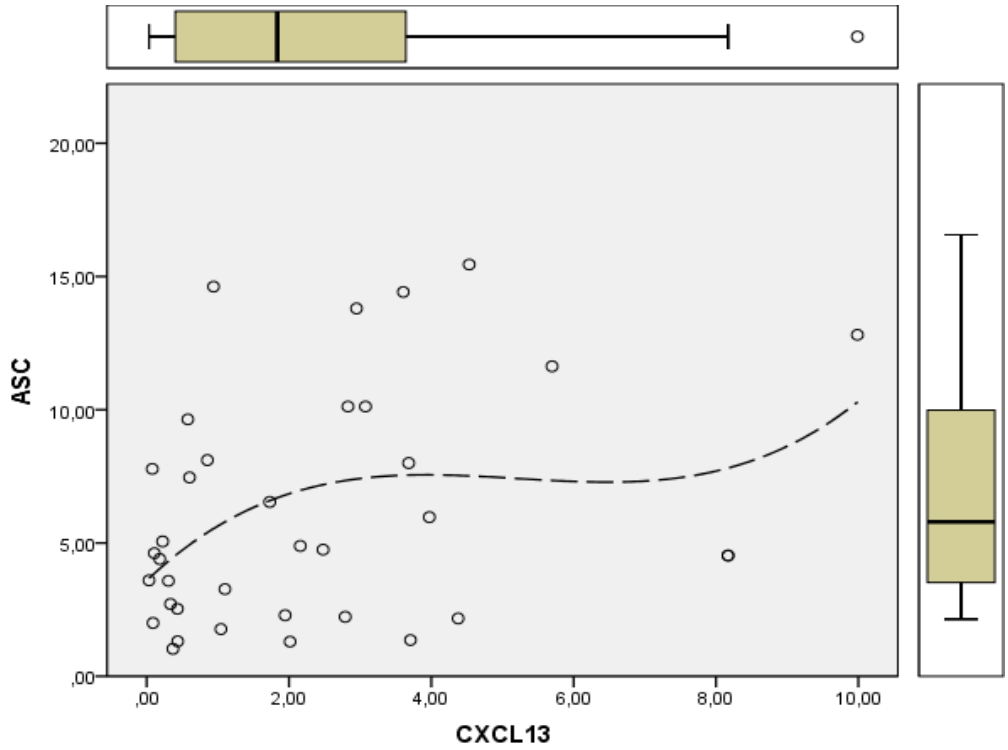

Figure 4. Cubic Table of the Relationship Between CXCL-13 and ASC

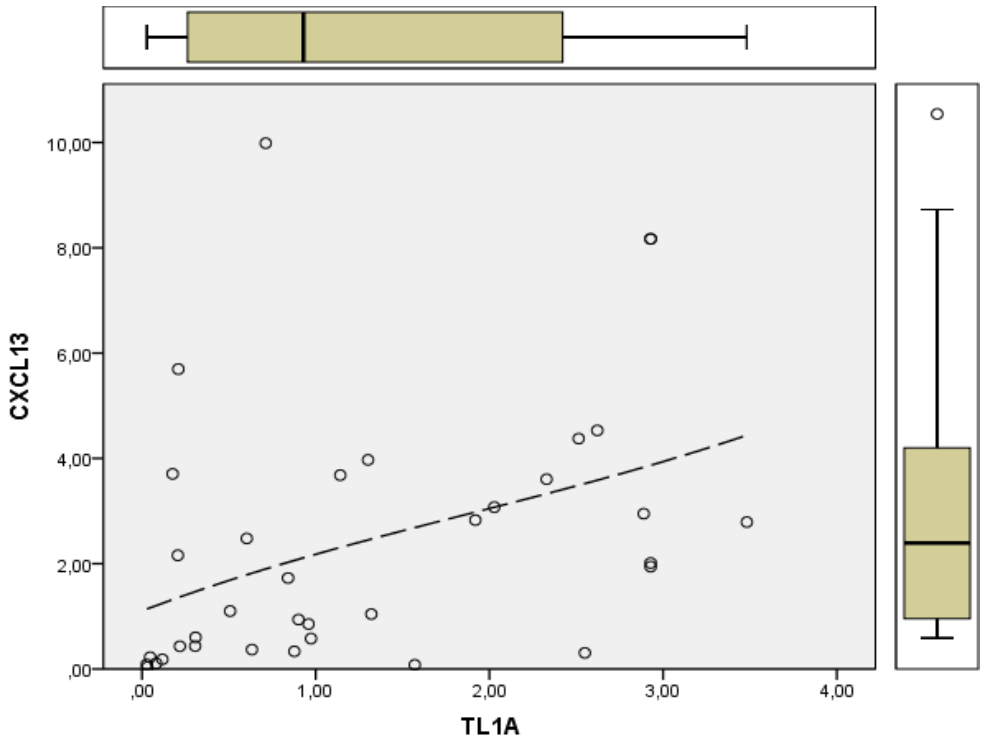

Figure 5. Cubic Table of the Relationship Between TL-1a and CXCL-13

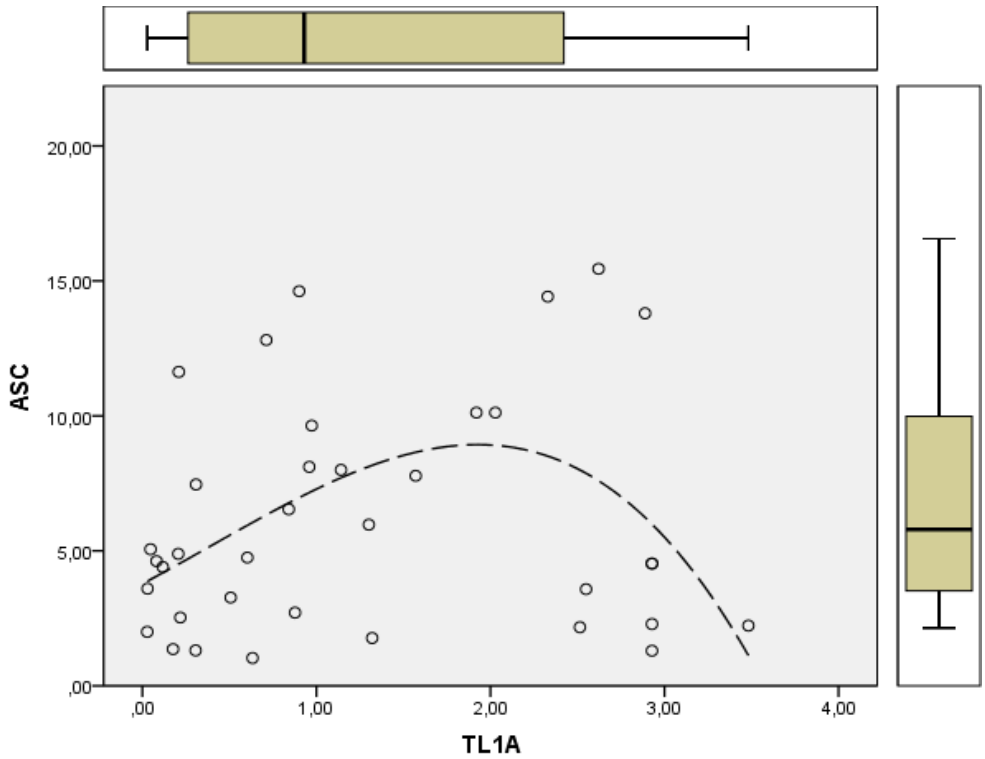

Figure 6. Cubic Table of the Relationship Between TL-1a and ASC

DISCUSSION

In addition to common variants, the novel variant such as 334-335 DelG (P. Glu112fs, C. 334-335delg) (27), K447M (P.LYS447MET, C.1340 A>T) (28), and rare variant such as A89T (p.Ala89Thr, c.265G>A) (29), E167D (30), 761_764dupCCGCp.Asn256Argfs70, c.761_ 764 dup CCGC (31), S288Y (P.SER863TYR, C.863 C>A) (32) were detected in patients with FMF.

It was reported that a case with Klippel-Feil Syndrome, bilateral Sprengel Deformity, congenital unilateral renal agenesis and a heterozygous mutation M680I(G>C) in the MEFV gene should be followed for kidney failure during her life for

amyloidosis risk (33). Patients with hereditary disease that can affect the kidney, if FMF is accompanied, closer monitoring is required for chronic kidney damage. Also it was reported that because the FMF patients with chest pain and at least one MEFV gene variant have increased risk for cardiac problems, these patients should be routinely followed up for cardiac problems (34). The understanding of both the contribution of the specific variant to clinical findings of FMF and genotype-phenotype correlation are important (1).

The symptoms of FMF can be confused with many diseases. Although clinical, laboratory and genetic assays help in making a diagnosis, there is no single diagnostic test that allows a definitive diagnosis. For this reason, it is very important to develop new markers and tests that will help to make an accurate diagnosis in the early period. That's why we planned to work now. There was a significant relationship between fever and CXCL-13 mRNA expression level ($p = 0.03$). However, there was no significant relationship between CXCL-13 mRNA expression level and both abdominal pain and joint pain ($p = 0.27$ and $p = 1.00$, respectively). According to this result, the increase in CXCL-13 mRNA expression level causes fever in patients and does not affect the occurrence of abdominal pain and joint pain symptoms.

Grossman et al. has shown that regular use of Colchicine for at least 12 months cause significant reduction in the symptoms of patients (35). In our study, a significant relationship was found with colchicine year and colchicine response in accordance with the literature. According to this result, colchicine treatment has been shown to be effective and colchicine is an effective drug in the treatment of FMF ($p = 0.00$).

Delibas et al. shown that the serum amyloid A(SAA) level was highest in the M694V gene mutation and erysipelas-like rash was more common than other mutations (36). In a study performed on gastric amyloidosis by Said et al., endoscopic evaluation revealed erythema of the gastric mucosa in some of the patients and amyloid deposition was the most common in the muscularis mucosa (37).

In our study, a significant relationship was found between erythema and Serum Amyloid A levels ($p = 0.05$). Although this suggests that erythema may be secondary to amyloid deposition in the skin, further studies are needed to fully explain this condition.

Significant relationship between symptom year and parental consanguinity was determined. According to this result, the symptoms of patients with familial mutation history were thought to start earlier than those with spontaneous mutations and the duration of symptoms was longer ($p = 0.01$).

Kasifoglu and colleagues in the study of FMF patients with end-stage renal failure in the family history of amyloidosis and end-stage renal failure revealed a significantly higher (38). In our current study, a significant relationship was found between family history and chronic kidney disease ($p = 0.04$). It is thought that the symptoms of familial mutation begin early and the risk of chronic kidney disease due to amyloid accumulation is increased in patients with a family history.

Yu et al. found that CRP and SAA levels of patients with sepsis were significantly higher than those without sepsis (39). In a study by Schellekens et al. found that CRP, SAA and leukocyte count were significantly increased in patients with acute appendicitis (40). In our study, there was a significant relationship between serum amyloid A level and CRP level and leukocyte count ($p = 0.00$). This shows us that SAA level is a good parameter for evaluating the systemic inflammatory response.

In the literature, to our knowledge only one study of ASC mRNA gene expression level in FMF patients has been found. In a study of 165 FMF patients in 2012, expression level of ASC mRNA was found to be significantly higher in MEFV gene mutation positive patients than negative, but there was no significant difference between the expression levels of ASC mRNA between MEFV mutation positive groups (41). Consistent with this study, ASC mRNA expression level was increased in the patient group compared to the control group, but this increase was not statistically significant ($p = 0.79$) (Table 3). This shows that ASC may play a role in the pathogenesis of FMF.

The best of our knowledge, there has not been any study about the CXCL-13 and TL-1a mRNA expression levels in patients with FMF. In the current study, we found that both CXCL-13 and TL-1a expression levels increased significantly in the patient group compared to the control group ($p = 0.03$; $p = 0.02$) (Table 3). This shows that CXCL-13 and TL-1a gene may have an important function in the pathogenesis of FMF disease.

As a result of this study; It may be said that ASC, TL-1a and CXCL-13 gene expressions were related to each other, that one could be the precursor or the product of the next step in the biochemical pathway and that these genes, their precursors or products could be targeted in the treatment of the disease. However, further studies are needed to better clarify the subject and to better manage the treatment strategy of the disease.

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RESEARCH ARTICLE

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The Relationship between Smoking Status, Carbon Monoxide Levels and Quality of Life, Disease Characteristics in Inflammatory Bowel Diseases

ABSTRACT

Objective: The aim of the study was to analyze the relationship between smoking status and exhaled carbon monoxide (E-CO) levels, quality of life, and disease characteristics in patients with inflammatory bowel disease.

Method: The demographic and disease characteristics and smoking status of 121 patients with inflammatory bowel disease who presented our hospital between 01.12.2020 and 01.03.2021 were investigated. After the first follow-up, the E-CO levels of these participants were measured every four consecutive weeks. The mean of these E-CO readings was accepted as the main E-CO value. After one month after their first application SF-36 Quality of Life Scale was applied. The relationship between these variables was investigated.

Results: The mean age of the participants was 42.06±14.9 years, and 36.3% were active smokers. While patients with Crohn's disease (CD) exhibited a higher smoking rate, smokers with ulcerative colitis (UC) registered significantly higher mean CO ppm readings ($p<0.05$). The general health components of smoker UC patients were higher than those of non-smokers ($p<0.05$). A weak correlation was determined between mean number of cigarettes smoked per day, mean CO ppm, Fagerström Nicotine Dependency Test (FNDT), package year, and the physical and mental components of SF-36 in the UC group ($p<0.05$). There was a weak negative correlation between mental components and mean E-CO in the CD group ($p=0.027$). No difference was observed in the non-smoker group between participants exposed to second-hand smoke and those with no such exposure ($p>0.05$).

Conclusion: Our results revealed that smoking has a weak positive effect on the quality of health in patients with UC, but no effect on patients with CD.

Keywords: Carbon Monoxide, Crohn's Disease, Inflammatory Bowel Disease, SF-36, Smoking, Ulcerative Colitis.

İnflamatuar Bağırsak Hastalıklarında Sigara İçme Durumu, Karbon Monoksit Düzeyleri ile Yaşam Kalitesi ve Hastalık Özellikleri Arasındaki İlişki

ÖZET

Amaç: Bu çalışmada inflamatuar barsak hastalığı olan hastalarda sigara içme durumu ile ekshale edilen karbon monoksit (E-CO) düzeyleri, yaşam kalitesi ve hastalık özellikleri arasındaki ilişkiyi incelenmiştir.

Gereç ve Yöntem: Hastanemize 01.12.2020-01.03.2021 tarihleri arasında başvuran inflamatuar bağırsak hastalığı (İBH) olan 121 hastanın demografik, hastalık özellikleri ve sigara içme durumları araştırıldı. İlk takipten sonra bu katılımcıların (E-CO) seviyeleri birbirini izleyen dört haftada bir ölçüldü. Bu E-CO okumalarının ortalaması, ana E-CO değeri olarak kabul edildi. İlk uygulamadan bir ay sonra SF-36 Yaşam Kalitesi Ölçeği uygulanarak değişkenler arasındaki ilişki araştırıldı.

Bulgular: Katılımcıların yaş ortalaması 42,06±14,9 yıl olup, %36,3'ü aktif olarak sigara içiyordu. Crohn hastalığı (CH) olan hastalar daha yüksek sigara içme oranı sergilerken, ülseratif kolitli (ÜK) sigara içenlerde anlamlı olarak daha yüksek ortalama CO ppm değerleri bulunmuştur ($p<0,05$). Sigara içen ÜK hastalarının genel sağlık bileşenleri içmeyenlere göre daha yüksekti ($p<0,05$). ÜK grubunda günlük içilen ortalama sigara sayısı, ortalama CO ppm, Fagerström Nikotin Bağımlılık Testi (FNBT), paket yılı ile SF-36'nın fiziksel ve mental bileşenleri arasında zayıf korelasyon saptandı ($p<0,05$). CH grubunda mental bileşenler ile ortalama E-CO arasında zayıf negatif korelasyon vardı ($p=0,027$). Sigara içmeyen grupta pasif içiciliğe maruz kalan katılımcılar ile böyle bir maruziyeti olmayanlar arasında fark gözlenmedi ($p>0,05$).

Sonuç: Sonuçlarımız, sigara içmenin ÜK tanılı hastalarda sağlık kalitesi üzerinde zayıf bir pozitif etkiye sahip olduğunu, ancak Crohn hastalarında herhangi bir etkisinin olmadığını ortaya koydu.

Anahtar Kelimeler: Crohn Hastalığı, İnflamatuar Barsak Hastalığı, Karbon Monoksit, SF-36, Sigara, Ülseratif Kolit.

INTRODUCTION

Inflammatory bowel disease (IBD), the two most important variants of which are ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory condition of the gastrointestinal tract. The disease is believed to result from both genetic predisposition and environmental factors leading to an immune response associated with the gut (1). The prevalence of IBD, which peaks most frequently between the ages of 20 and 40 or around the age of 60, has increased worldwide in the last 10 years. Approximately 0.2% of the European population is diagnosed with IBD, while Asia is the continent with the highest number of IBD patients (2). The incidence of UC in a multicenter study from Turkey in 2009 was 4.4 per 100,000, with a reported incidence of CD of 2.2 per 100,000 (3).

One of the most important environmental factors affecting the disease is smoking, the first reports concerning which date back to the 1980s (4). The relationship between smoking and IBD is a complex one. Smoking adversely affects the composition of the lumen (mucus and microbiota), mucosal structure, and the immunological response in the gastrointestinal system (GIS) (5, 6). Some studies have observed a high risk of CD and a low risk of UC in smokers with IBD compared to non-smokers (7, 8). Disease activity, postoperative exacerbation, and first and second surgery requirements in CD are lower among smokers who quit, being almost at the same levels as non-smokers (9). In contrast, the protective effect of smoking on UC is thought to be temporary. An individual who quits smoking has a greater risk of developing UC increased compared to patients who have never smoked. At the same time, smoking may not only protect against the development of UC but may also improve its clinical course. Smokers have been observed to experience fewer disease recurrences, lower steroid or immunosuppressive therapy requirements, fewer hospitalizations, and fewer colectomy procedures (10).

The quality of life of IBD patients is seriously adversely affected in physical, psychological, and social terms. Lengthy and painful disease exacerbation phases (abdominal pain, blood in the stool, etc.) affect most daily GIS functions, but especially nutrition. Blood tests and invasive interventional diagnostic methods such as colonoscopy and sigmoidoscopic biopsy are often needed in these cases to evaluate the exacerbation. Anti-inflammatory therapy used to suppress frequent, severe, and painful GIS symptoms can cause severe side effects (11). Tobacco products (cigarettes, pipe tobacco, etc.) contain more than 4000 toxic and carcinogenic chemicals and produce severe adverse effects on users' quality of life, and smoking will also inevitably impact the quality of life of patients with IBD (12). The deleterious effects on the GIS of numerous harmful chemicals in cigarette smoke have been isolated and reported in published studies. Most of the studies examining the relationship between smoking and IBD so far have focused on daily nicotine intake (13). Nicotine exerts an immunomodulatory effect mediated by the activation of nicotinic receptors of alpha-7 in immune cells such as macrophages and dendritic cells. Recent studies suggest that CO as well as nicotine from cigarettes may have an impact on the clinical features of IBD patients. It was thought that the increase in the CO level had an anti-inflammatory effect and therefore had effective results in the clinic of IBD. CO level affects the maturation of dendritic cells and reduces antigen presentation to the immune system. It also exerts an anti-inflammatory effect by reducing leucocyte migration (14). So far various studies investigated the effect of smoking on quality of life either with self-reports or with cotinine levels (Main nicotine metabolite) in IBD patients. However, there have been no detailed studies investigating the relationship between the quality of life of IBD patients and smoking and CO levels measured in expiratory air, components of cigarette mainstream smoke.

The principal aim of this study is to investigate the relationship between smoking and E-CO measurements, disease characteristics, and quality of life in patients with UC and CD.

MATERIAL AND METHODS

Design of the Study: This case-control study involved 121 patients with IBD who presented to the Ondokuz Mayis Faculty of Medicine between December 2020 and March 2021. Using G-Power analyses before commencement we calculated that a total of 110 cases would be sufficient for the sample.

Patients older than 18, volunteering to participate, and previously diagnosed with IBD were included in the study (n=125). A questionnaire investigating the cases' sociodemographic characteristics and disease features was completed during face-to-face interviews at the first application. Also, the first E-CO measurement is performed at this time, and rendezvous was given to every participant for the next following three weeks. At these three meetings, the participants' E-CO measurements were repeated and their smoking status is checked. Four subjects who missed their rendezvous were also excluded from the study. The mean of these four E-CO readings is accepted as the main E-CO value for the participants. Quality of life was then evaluated using the SF-36 test. The cases' smoking status was determined, and smokers' addiction levels were graded using the Fagerström Nicotine Dependence Test (FNDT). Pack/year values were also calculated. E-CO levels were measured after all participants had completed the SF-36 quality of life assessment scale.

Individuals who have smoked more than 100 cigarettes over six months or longer are regarded as smokers. Participants who had quit smoking for more than a year were regarded as non-smokers in two-group analyses in the present study. Two groups were established in terms of smoking status (smokers and non-smokers). Individuals who had quit smoking less than one year previously were excluded from the study (n= 6).

Tools

E-CO measurements: All exhaled breath CO measurements were performed using a Tabataba V2 device, an analyzer that measures CO in exhaled breath. This is used to calculate the amount of CO intoxication in both smokers and non-smokers. The CO measurement range for the device is 0-400 ppm, and the accuracy is ±1 ppm. The relationship between ppm and the number of cigarettes smoked is 0-5 ppm – Non-smoker, 5-10 ppm - Passive smoker or light smoker, 10-15 ppm - Frequent smoker, 15-25 ppm - Heavy smoker, and 25-50 ppm - Very heavy smoker.

SF-36: The SF-36 scale was first designed by Ware et al. in 1992 (15). It consists of 36 questions and two main components (Physical and Mental Health) with eight sub-dimensions, which measure the quality of life within the previous month. The Physical component (PC) consists of physical functioning, role limitation due to physical problems, bodily pain, and general health perception sub-dimensions, while the Mental component (MC) consists of social functionality, role limitation due to emotional problems, vitality [energy], and mental health sub-dimensions. We analyzed all of the sub-dimensions individually and then combined them to evaluate the Physical (PC) and Mental Health (MC) components' total scores. Possible scores range from 0-100, with 100 representing the best score for each sub-dimension. The validity and reliability of the SF-36 in Turkish have been previously investigated (16).

Fagerstrom Nicotine Dependency Test (FNDT): The Fagerstrom Nicotine Dependency Test is a self-assessment scale developed by Heatherton et al. consisting of six questions, scored between 0 and 10, used to assess the risk of physical dependence on nicotine (17). The validity and reliability of the FNDT in Turkish were studied by Uysal et al. (18). Scores of 0-2 are interpreted as indicating low-level addiction, 3-7 as medium-level addiction, and 8-10 as high-level addiction.

Statistical Analyses: All the data were uploaded onto Statistical Package for Social Sciences version 22 software. The homogeneity of all the variables was tested using the Kolmogorov-Smirnov test. The SF-36, FNDT, and SF-36 scores were adopted as independent variables. The type of IBD and smoking status were adopted as dependent variables. The statistical relationships between these variables were investigated using the Chi-square, bivariate correlation, and Independent-Samples t-tests. A p level <0.05 was regarded as statistically significant.

Ethics: Approval for the study was granted by the Ondokuz Mayis University Ethical Committee before commencement. The aim of the study was explained to all the participants, and their written consent was obtained before any data were collected.

RESULTS

One hundred twenty-one patients with IBD were included in the study, of whom 39.7% were women (n= 48), 71.9% (n= 34) were married, and 30.7% (n= 36) were educated to the university level or higher. The mean age of the participants was 42.06±14.9 years. Seventy-eight (64.5%) cases were diagnosed with UC and 48 (39.7%) with CD. Participants had been diagnosed with IBD a mean 6.32±5.4 years previously, and their mean age at diagnosis was 35.12±13.08 years. A comparison of the participants' demographic data according to their diagnoses is shown in Table 1.

Table 1. The demographic variables of the patients with UC and CD

Variable	UC	CD n= 45	p
Gender			
Male	44 (58%)	38 (64%)	x^2= 0.922
Female	32 (42%)	17 (36%)	p= 0.203
Mean Age	43.22±12.5	37.68±13.1	t= 2.936 p= 0.004
Marital Status			
Single	12 (19.8%)	14 (34%)	x^2= 8.493 p= 0.003
Married	61 (80.2%)	29 (62%)	
Divorced/Widowed	3 (4.9%)	2 (4%)	
Mean Years of Education	8.12±1.1	8.08±0.9	t= 0.631 p= 0.27

Smoking status, mean E-CO measurements, the mean number of cigarettes smoked per day, FNDT, and pack/year values among the active smokers with UC and CD are shown in Table 2. Analysis showed that 36.3% of the study population were smokers and that 68.2% of the active smokers had previously quit attempts at least once (min= 1, max= 11). Male participants (n= 29, 65.9%) had a

higher smoking rate than women (n= 15, 34.1%) (x^2=17.250, p<0.001). There was no statistical difference between the mean values of each four E-CO readings in both smokers (F=0.235) and non-smokers (F=0.852)

Disease characteristics and features according to smoking status in IBD cases with different diagnoses are shown in Table 3. No statistically significant correlation was observed between gender and diagnosis of IBD. However, patients with CD had a higher proportion of smokers than patients with UC (28.2% versus 44.4%, x^2=6.313, p=0.017).

Table 2. The smoking features and mean E-CO values of the patients with UC and CD

Variables	UC n (%)	CD n (%)	p
Smoking Status			
Smoker	22 (27.2%)	20 (44%)	
Non-Smoker			
Ex-Smoker	27 (37.0%)	17 (38%)	x^2=11.991, p=0.002
Never Smoked	27 (37.0%)	8 (18%)	
FNDT*	3.70±1.3	2.71±2.2	t=3.903, p<0.001
The mean number of cigarettes smoked in a day	8.04±1.3	6.52±14.5	t=6.255, p<0.001
Package/year	7.17±14.6	4.30±0.9	t=1.694, p=0.091
Mean CO ppm	6.7±7.5	4.09±4.8	t=3.453, p=0.001

***FNDT= Fagerström Nicotine Dependency Test**

Table 3. Disease features of the patients with UC and CD according to smoking status

Variable	**UC** n= 76		t, p	**CD** n= 45		t, p
	Smoker n= 22	Non-Smoker n= 54		Smoker n= 20	Non-Smoker n= 25	
Age (years)	40.12±9.9	44.15±13.0	1.587, 0.072	37.06±11.1	38.7±15.2	0.322, 0.462
CO ppm (mean)	12.7±1.2	1.41±0.18	11.746, <0.001	10.4±6.1	1.2±0.25	10.58, <0.001
Duration of the Disease (years)	7.85±4.1	7.23±2.1	1.822, 0.07	3.22±3.1	4.8±3.0	1.125, 0.347
Age at Diagnosis (years)	33.28±10.8	37.98±13.8	5.897, 0.010	34.3±11.1	35.0±15.7	0.227, 0.821
Mean Number of Exacerbations in the Previous Two Years	1.45±2.7	2.6±3.1	1.520, 0.122	3.64±5.9	1.89±2.57	1.928, 0.052
Mean Number of Hospital Admissions in the Previous Two Years	10.7±9.8	7.55±5.6	3.411, <0.001	9.78±1.8	8.06±4.1	2.211, 0.03
Number of Hospitalizations	0.41±0.8	0.76±1.4	1.570, 0.118	1.28±3.6	1.68±2.2	0.177, 0.368
Mean Number of Surgical Operations	0.04±0.6	0.28±1.1	2.735, 0.014	1.09±1.3	0.86±1.2	1.857, 0.058
History of Surgery (n, %)	3, 9.1%	2, 1.7%	4.741, p=0.124	13 (52 %)	12 (48 %)	2.795, 0.061
Treatment (n, %)			x^2, p			x^2, p
Immunosuppressive	6, 27.3%	28, 47.5%	5.655, 0.017	8 (50%)	8 (50%)	0.255, 0.501
Anti-TNF	7, 31.8%	10, 16.9%	4.327, 0.032	12 (57.1%)	9 (42.9%)	5.186, 0.005
Corticosteroid	2, 4.5%	11, 18.6	1.008, 0.152	4 (50%)	4 (50%)	0.511, 0.652
5-ASA	17, 77.3%	51, 86.4%	1.099,0.102	11 (35.5%)	20 (64.5%)	

Seventeen (38.6%) active smokers stated that they wished to quit smoking within the following six months. No significant relationship was found between willingness to quit smoking and disease diagnosis (x^2=1.014). Twenty-three non-smokers (29.9%) reported being exposed to second-hand smoke during the day; 9.2% of the participants reported being exposed to second-hand smoke at home, 16.8% at work, and 29% in their social environment. The mean E-CO measurements (1.77±0.88 ppm) of these non-smokers who reported exposure to second-hand smoke did not differ significantly from those of the other non-smokers (1.75±0.6) (t=0.087).

The Quality of Life of the Patients with UC and CD: A comparison of the SF-36 sub-dimensions between the patients with UC and CD patients is presented in Table 4. No difference was observed between the groups' mean scores from the eight SF-36 sub-dimensions except for general health perception, on which the patients with UC registered better results than those with CD. There was no significant difference in mean total SF-36 MC and PC scores between the UC and CD groups.

Analysis of the SF-36 sub-dimensions among the patients with UC showed that male patients achieved better physical functioning subdimension scores than women (86.5±20.6 vs

77.9±23.9, respectively, t=2.406, p=0.015). Men with CD also registered better physical functioning sub-dimension scores than women (87.6±20.9 vs 78.0±20.9, respectively, t=2.340, p=0.021).

Table 4. A comparison of the SF-36 sub-dimensions of SF-36 between the UC and CD groups

SF-36 Subdimensions (mean)		UC (n=76)	CD (n=45)	t, p
Physical Component	Physical Functioning	82.9±22.4	84.2±20.1	0.451, 0.653
	Physical Role Limitations	63.2±47.4	58.7±48.7	0.741, 459
	General Health Perceptions	61.6±24.9	54.8±28.1	2.045, 0.042
	Bodily Pain	64.5±31.3	72.6±84.8	1.091, 0.276
	Total Physical Component Score	272.4±102.5	270.4±121.7	0.104, 0,917
Mental Component	Mental Health	59.9±16.0	59.6±16.4	0.137, 0.891
	Social Function	68.9±27.3	65.4±26.4	1.206, 0.306
	Energy/Vitality	50.1±20.3	47.3±18.1	1.108, 0.269
	Emotional Role Limitations	69.1±46.3	72.6±44.2	0.608, 0.544
	Total Mental Score	248.0±83.1	245±84.5	0.201, 0.841

Relationships between Quality of Life, Mean Number of Cigarettes Smoked in Day FNDT, Package/Year, and E-CO Measurements: The SF-36 general health sub-dimension scores of smokers with UC (71.36±24.7) were significantly higher than those of the non-smoker UC group (58.7±24.2) (t=2.052, p=0.042). The mean total SF-36 PC scores were significantly higher among the smoker UC patients compared to the non-smokers (296.4±107.1 vs. 241.52±100.2, t=1.285, p=0.002). The mean total SF-36 MC scores were significantly higher in the smoker UC patients compared to the non-smokers (285.1±87.4 vs. 231.81±101.2, t=1.665, p=0.005). Correlations between mean number of cigarettes smoked per day, FNDT, package/year, and E-CO measurements, and the PC and MC of the SF-36 in patients with UC are presented in Table 5. A weak correlation was observed between E-CO ppm, the mean number of cigarettes smoked per day, FNDT, and package year, and the mean total SF-36 MC and PC scores ($p<0.05$).

Table 5. Correlations between FNDT, Package/year, and E-CO measurements and the SF-36 Physical and Mental dimensions in the UC group

Variable	PC-SF-36	MC-SF-36	CO ppm	FNDT	The mean number of cigarettes smoked per day	Package/year
PC-SF-36	1					
MC-SF-36	0.713*, 0.001**	1				
Mean CO ppm	0.163*, 0.038**	0.164*, 0.037**	1			
FNDT	0.237*, 0.002**	0.219*, 0.005**	0.754*, 0.001**			
The mean number of cigarettes smoked per day	0.314* 0.001**	0.298* 0.002**	0.785* 0.001**	0.755* 0.001**	1	
Package/year	0.185*, 0.019**	0.187*, 0.017**	0.603*, 0.001**	0.575*, 0.001**	0.561* 0.001**	1
r=* **p=****						

The mean scores of the eight SF-36 sub-dimensions in the smoker CD group did not differ significantly from those of the non-smokers ($p>0.05$). There was a weak negative correlation between MC and E-CO in the CD group (p=0.027). Correlations between the mean number of cigarettes smoked per day, FNDT, package/year, and E-CO measurements and the Physical and Mental dimensions of the SF-36 in the CD group are shown in Table 6.

Table 6. Correlations between FNDT, Package/year, and E-CO measurements and SF-36 Physical and Mental dimensions in the CD group

Variable	PC-SF-36	MC-SF-36	CO ppm	FNDT	The mean number of cigarettes smoked in a day	Package/year
PC-SF-36	1					
MC-SF-36	0.582*, 0.001**	1				
Mean CO ppm	0.175*, 0.081**	-0.199*, 0.027**	1			
FNDT	-0.145*, 0.150**	-0.139*, 0.169**	0.810*, 0.001**	1		
The mean number of cigarettes smoked in a day	-0.122 0.087	-0.250 0.090	0.658 0.001	0.712 0.001	1	
Package/year	-0.060*, 0.551**	-0.023*, 0.818**	0.521*, 0.001***	0.670*, 0.001**	0.590*, 0.001**	1
r=* **p=****						

DISCUSSION

Our study is the first in the literature to investigate the mean E-CO level and quality of life and clinical characteristics of IBD patients as far as we know. In this context, some striking results have been achieved. Smoking rates were quite high in our sample (34.7%), with 44.4% of patients with CD and 29.3% of those with UC being active smokers. Although smoking rates were higher among the patients with CD, the UC group exhibited higher FNDT, pack/year, and mean CO ppm measurement values. Smokers with UC and CD both had much higher mean E-CO measurements than non-smokers (9-fold and 8.9-fold, respectively). A study of 1098 cases of IBD reported a smoking rate of 10%, 88.2% of these being patients with CD (Scoville et al., 2020). Also similar to our results, a cohort study of 1203 participants by Lunney et al. reported a higher frequency of smoking in patients with CD (19).

The results of this study indicate that GIS symptoms commenced four years later in non-smoker patients with UC. Smokers with UC also experienced less frequent disease exacerbation, had fewer hospital admissions, and needed fewer surgical operations in the previous two years. However, they also had greater immunosuppressive requirements and received more anti-TNF therapies. There are conflicting reports regarding the clinical course in patients with UC who smoke. One meta-analysis study concluded that smoking is a protective factor in terms of clinical outcomes of UC (20). However, this positive effect disappears when a smoker with UC quits, and symptoms peak within one year (21). It has also been emphasized that this clinical deterioration is dose-dependent and that heavier smokers are more susceptible to the risk of disease exacerbation after cessation compared to light smokers (22). We, therefore, excluded patients who had quit smoking one year previously so that they would not affect our results. There are also conflicting reports concerning the effect of smoking on the frequency of surgical procedures and the risk of postoperative complications. Some studies have reported a significant decrease in the number of surgical procedures, while some have observed no effect, and others have shown a significant increase (23-25).

The results of this study indicate no significant difference in terms of clinical outcomes. Like smoker participants of UC, smoker CD patients had higher numbers of hospital visits in the previous two years compared to non-smokers. Previous studies have described smoking as the most potent environmental factor in the development and clinical manifestation of CD (26, 27). Studies have estimated that smoking increases the incidence of CD 1.8 to 4.6-fold (28, 29). Smoking cessation has therefore been recommended as the primary therapeutic approach in CD (30). Despite inconsistent reports having been published (27), the risk of exacerbation and clinical symptom severity decreases two-fold after quitting among smokers with CD patients in the following years (31). A smoking cessation intervention study by Cosnes et al. reported that the clinical course improved in the 474 smokers with CD (with a quit rate of 14%) who had quit smoking for more than one year, while the number of attacks was significantly lower (12). Other follow-up studies of the improvement in the clinical course of CD in patients who quit smoking have confirmed these data (32, 33).

The findings of this study revealed no difference between the quality of life of patients with UC and CD in terms of the eight sub-dimensions of the SF-36. However, other studies have observed a higher quality of life in patients with UC than in those with CD (34, 35). Our results indicated a weak and positive relationship between

the Physical and Mental components of the SF-36 and the mean number of cigarettes smoked per day, FNDT scores, pack/year values, and mean E-CO measurements in the UC group. Among the SF-36 sub-dimensions, only the mean General Health Perception was higher in the patients with UC who smoked than in the non-smokers. We also determined a weak negative correlation between E-CO measurements and MC of the SF-36 in the CD group patients. Other studies have observed significantly lower quality of life in active smokers with CD (21, 36). Our results may have been affected by the demographic and smoking features of the study population. The CD cases in our sample were on average five years younger and smoked less than the UC cases. In addition, the SF-36, a common clinical scale, was used to evaluate the quality of life in the present study. This scale is also affected by other comorbid chronic diseases. The effects of other chronic diseases in our cases could not, therefore, be eliminated in the analyses. Other quality-of-life questionnaires are available for clinical follow-up of IBD patients, and studies using these surveys may produce different results (37).

The study results revealed no relationship between second-hand smoking and quality of life in patients with IBD. The World Health Organization notes that there is no safe range for exposure to secondhand smoke (38). Our scan of the literature revealed no randomized controlled trials showing a direct relationship between passive exposure to cigarette smoke and IBD. While nearly 40% of smokers in the present study stated that they were considering quitting, no difference was found between the two diseases in terms of motivation. Similarly, a study conducted with CD patients emphasized that although nearly 90% of the patients had thought about quitting smoking, less than 30% were willing to participate in a free smoking cessation program (39).

Limitations and Directions/Suggestions for Future Research: There are several strengths and weaknesses to this investigation of the effect of smoking on the quality of life and disease characteristics of patients with IBD. Although our university is the most important reference center in the region, this research is not a descriptive study aimed at determining the true proportion of smokers among patients with IBD in our region. As described above, the two most important agents in smoke that affect the clinical outcomes of IBD are believed to be nicotine and CO (40, 41). Patients' exposure to nicotine was evaluated using the mean number of cigarettes smoked per day, FNDT, and pack/year criteria. However, these are declarative parameters that do not directly reflect total plasma nicotine levels. Cigarettes smoked per day provide a crude estimation of the nicotine consumed in a day as the nicotine amount of each cigarette brand differs (light cigarettes etc.). In contrast to this phenomenon, E-CO measurements provide an objective variable for determining exposure to smoke which can be measured very easily. However, it is a disadvantageous situation that E-CO measurements provide short-term information about the level of cigarettes smoked as the mean half-life of exhaled CO in smokers is approximately five hours (42). Also, E-CO measurements can be affected by numerous different environmental factors (indoor smoking, being in well-ventilated areas, etc.). To minimize this disadvantageous situation, we performed four measurements each week to monitor these readings for a longer period. In this way, we had the advantage of evaluating average measures of CO ppm in every participant. Our results showed us that there was no difference in the E-CO levels of the study participants during these four weeks, regardless of their smoking status. Also, the clinical features of UC and CD can differ significantly, depending on the anatomical site of the inflammatory process in the GIS (43). However, since colonoscopy was not applied during the study, these sites of involvement could not be determined exactly. These factors may have influenced our results. In addition, one of the most important limitations of our study is that the disease activity of patients, which may affect the quality of life, has not been evaluated. Therefore, we believe that it will be useful to evaluate this factor in future studies.

In conclusion, a weak, positive correlation was found between quality of life in patients with UC and the mean number of cigarettes smoked per day, FNDT, pack/year, and E-CO levels, also there was a negative correlation between E-CO measurements and MC in patients with CD.

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RESEARCH ARTICLE

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Self-Compassion in Depression and Anxiety Disorders

ABSTRACT

Objective: The purpose of this study was to determine the levels of self-compassion, psychological well-being, and self-esteem in patients suffering from depression and anxiety disorders, as well as the relationship between them.

Method: The study included 100 patients with depressive disorders and 100 patients with anxiety disorders who applied to Afyonkarahisar Health Sciences University Psychiatry Outpatient Clinic and agreed voluntarily to participate in the study, and also 100 healthy controls. Sociodemographic data form, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Psychological Well-Being Scale (PWBS), Self-Compassion Scale (SCS) and Rosenberg Self-Esteem Scale (RSES) were applied to the participants.

Results: According to the findings of the scales applied to the groups; there was a statistically significant difference between the groups in terms of well-being, self-esteem, and self-understanding ($p<0.001$). The distribution of median values (m(Q1-Q3)) of self-understanding levels by groups is as follows; depression group (60(48-72)), anxiety disorders group (71(58-84)) and healthy control group (93(88-97)). In the Pearson correlation analysis, it was determined that self-compassion showed the strongest correlation with self-esteem in the depression group ($r=0.594$, $p<0.001$).

Conclusion: As a result of the strong correlations between psychological well-being and self-esteem, it has been determined that it is critical to prioritize self-compassion in depression and anxiety disorders accompanied by depressive symptoms.

Keywords: Anxiety Disorders, Depression, Self-Esteem, Self-Compassion.

Depresyon ve Anksiyete Bozukluklarında Öz-Anlayış

ÖZET

Amaç: Bu çalışma depresyon ve anksiyete bozuklukları hastalarında öz anlayış, psikolojik iyi oluş ve benlik saygısı düzeylerini tespit etmek ve aralarındaki ilişkiyi belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: Afyonkarahisar Sağlık Bilimleri Üniversitesi Psikiyatri polikliniğine ayaktan başvuran ve çalışmaya gönüllü olarak katılmayı kabul eden 100 depresyon, 100 anksiyete bozukluğu hastası ile 100 sağlıklı kontrol çalışmaya dahil edilmiştir. Katılımcılara; sosyodemografik veri formu, Beck Anksiyete Ölçeği (BAÖ), Beck Depresyon Ölçeği (BDÖ), Psikolojik İyi Oluş Ölçeği (PİOÖ), Öz Anlayış Ölçeği (ÖZAN) ve Rosenberg Benlik Saygısı Ölçeği (RBSÖ) uygulanmıştır.

Bulgular: Tüm gruplara uygulanan ölçeklerin analiz sonucuna göre; iyi oluş, benlik saygısı ve öz anlayış düzeylerinin gruplar arasında istatistiksel olarak anlamlı farklılığa sahip olduğu belirlendi ($p<0.001$). Öz anlayış düzeylerinin median değerlerinin (m(Q1-Q3)) gruplara göre dağılımı şu şekildedir; depresyon grubu (60(48-72)), anksiyete bozuklukları grubu (71(58-84)) ve sağlıklı kontrol grubu (93(88-97)). Pearson korelasyon analizinde öz anlayışın en güçlü korelasyonu depresyon grubundaki benlik saygısı ile gösterdiği tespit edildi ($r=0.594$, $p<0.001$).

Sonuç: Psikolojik iyi oluş ve benlik saygısı ile gösterdiği güçlü korelasyonlar sonucunda, depresyon ve depresif belirtilerin eşlik ettiği anksiyete bozukluklarında öz anlayışa öncelik verilmesinin önemli olduğu belirlenmiştir.

Anahtar Kelimeler: Anksiyete Bozuklukları, Depresyon, Öz Saygı, Öz Anlayış.

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INTRODUCTION

Self-compassion is being kind and understanding towards oneself in difficult experiences. It is an emotional regulation strategy that makes the individual conscious of pain and realizes suffering as a universal human experience rather than seeing it as the individual's own. Self-compassion, which requires an individual to evaluate their emotions and thoughts without judgment, is made up of three major components: self-kindness, common humanity, and mindfulness (1). Self-compassionate people recognize that difficulties and mistakes are universal. As a result, the individual's mistakes and difficulties would not be viewed as personal failures, but rather as evidence of their own humanity (2).

It is known that coldness towards oneself, which is characterized by the intense negative components of self-compassion, has a strong relationship with psychopathology, especially with anxiety and depression, and with the various effects to quality of life (3,4). It has been observed that patients who are followed up with a diagnosis of major depression show a lower level of self-compassion even when they are in remission, compared to individuals who have never had depression (5). Furthermore, a study discovered that while depression predicted lack of self-compassion, depressive symptoms did not predict lack of self-compassion, and it was suggested that lack of self-compassion did not occur as a result of depression (6). Self-compassion is thought to protect individuals from depression and anxiety (7–9). As a result of a study with a small sample of 10 people with any of the diagnoses of anxiety disorder and depression, it was seen that all of the participants stated that they found self-compassion meaningful but had difficulty in realizing it (10). It has been revealed that treatments that aid in the development of self-compassion contribute to the reduction of depression symptoms (2).

Self-esteem, on the other hand, encompasses both positive and negative attitudes toward one's own. It is composed of the concepts of self-love and self-efficacy (11). Self-love arises when people evaluate themselves as good or bad. The concept of self-efficacy is the individual's feeling of seeing oneself as effective and in control. In other words, self-esteem stems from how people perceive themselves and how they perceive their abilities (12). Self-compassion is a source of positive self-respect, just like self-esteem. When people behave with understanding towards and perceive themselves as part of a whole, they feel more valuable, accepted and safe. There are also places where self-respect and self-compassion diverge. For example, individuals with high self-compassion have a lower rate of comparing themselves with other individuals than individuals with high self-esteem. That is, self-esteem is more concerned with comparing oneself with others, rather than being in a relationship with others (13,14). It has been suggested that self-esteem can predict self-compassion, although there are situations where they resemble and differ from each other. But the opposite is not true (15). There is a reciprocal relationship between self-esteem and depression and anxiety; low self-esteem can cause depression and anxiety, or it can be seen as a result of depression and anxiety (16).

Psychological well-being is defined as self-actualization and living a meaningful life in the face of existential difficulties such as establishing quality relationships (17). Evaluating the sub-dimensions of self-acceptance, environmental dominance, autonomy, positive relationships with others, life purpose and personal development together give information about the individual's psychological well-being (18). It is known that self-compassion correlates positively with well-being (19).

Individuals with high self-compassion are expected to have higher self-esteem, less exposure to stressful life events, high levels of psychological well-being, and low levels of depression and anxiety, according to the above theoretical information and related research. Although research on self-compassion has intensified in international literature in recent years, it has been noted that most of these studies have been conducted on non-clinical samples, and there are limited studies on this subject in our country. In the current study, our hypothesis is that there is a difference in terms of self-compassion in patients with depression and anxiety disorder, that there is lower self-compassion in the depression clinic than in the anxiety disorder. This has the same effect on self-esteem and psychological well-being levels at the same rate.

MATERIAL AND METHODS

The Ethics Committee Approval was obtained from the Afyonkarahisar Health Sciences University, Clinical Research Ethics Committee in 11/06/2020 with the number 2021/115. Written informed consent was prepared according to the principles of the Declaration of Helsinki before the study and obtained from all the participants included in the study. A total of 216 patients, aged between 18-65, who were admitted to the Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Psychiatry Outpatient Clinic, consecutively between 01.03.2021 and 01.09.2022, who were diagnosed with major depression or anxiety disorder according to the DSM-5 criteria and receiving outpatient treatment were included in the study. However, according to the exclusion criteria, 13 people were excluded from the study due to systemic disease and 3 people due to other psychiatric comorbidities, and the study was completed with the remaining 200

patients and 100 healthy controls who voluntarily agreed to participate in the study.

The study inclusion criteria were: being between 18 and 65 years of age and having a diagnosis of major depression or anxiety disorder and giving consent to participate in the study. ***Exclusion criteria were:*** having any of the chronic systemic diseases, comorbid psychiatric disorders, or severe neurological impairments, and also being pregnant at the time of the study.

SCID-5 (Structured Clinical Interview According to the DSM-5), a clinical interview structured according to the DSM-5 (Diagnostic and Statistical Manual for Psychiatric Disorders), which was published by the American Psychiatric Association in 2013, has been implemented. In addition; the sociodemographic data form, Beck Anxiety Inventory, Beck Depression Scale, Psychological Well-Being Scale, Self-Compassion Scale and Rosenberg Self-Esteem Scale were applied.

Instruments of Assessment

1. Sociodemographic data form: This form consists of questions including sociodemographic information such as age, gender, educational level, and job status.

2. Beck Anxiety Inventory: It is a self-report scale developed by Beck et al. (20) in 1988. The total score of the scale, which consists of 21 items, is used to determine the level of anxiety. The Turkish validity and reliability were evaluated by Ulusoy et al. (21).

3. Beck Depression Scale: This scale, which has a total score ranges from 0 to 63, is a self-assessment scale consisting of 21 items in 4-point Likert structure. The Turkish validity and reliability of this scale, which was developed by Beck et al. (22) in 1961, was performed by Hisli in 1989 (23).

4. Psychological Well-Being Scale: Consisting of eight items, the Psychological Well-Being Scale defines important elements of human function, from positive relationships to feelings of efficacy, to having a meaningful and purposeful life. The Turkish validity and reliability of the scale developed by Diener et al. (24) was performed by Telef (25).

5. Self-Compassion Scale: The original Self-Compassion Scale consists of 26 items and 6 subscales. Respondents in the scale are asked to rate how often they act in relation to the stated situation on a 5-point Likert-type scale ranging from “Almost never=1” to “Almost always=5”. The Turkish reliability and validity study of the Self-Compassion scale developed by Neff (26) was conducted by Deniz, Kesici and Sümer (27). The Turkish Self-Compassion Scale, unlike the original, shows a one-dimensional structure.

6. Rosenberg Self Esteem Scale: It was developed by Rosenberg (28) in 1965 as a one-dimensional scale with 10 items. The Turkish adaptation of the scale was made by Çuhadaroğlu (29).

Statistical Analysis

The obtained data were evaluated with the SPSS version 25 package program (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to determine the distribution characteristics of the variables. Continuous variables with normal distribution were presented as mean and standard deviation values. Non-normal variables were expressed as median and 25-75 percentile (Q1-Q3) values. Correlation between scales were analyzed using the Pearson correlation test. P value less than 0.05 was considered statistically significant.

RESULTS

A total of 300 volunteers, including 100 depression patients, 100 anxiety patients, and 100 healthy volunteers, were included in the study. The mean age of the depression group was 34.7±11.4, the anxiety group was 35.1±12.5, and the healthy control group was 31.6±8.9. Female participants comprised 76% of the depression group, 68% of the anxiety group, and 64% of the healthy control group. Other sociodemographic data of the groups are shown in Table 1.

When the results of the clinical scales applied to the groups were evaluated, it was observed that there was a statistically significant difference between all groups in all scales. The distribution of median values (m(Q1-Q3)) of self-understanding levels by groups is as follows; depression group (60(48-72)), anxiety disorders group (71(58-84)) and healthy control group (93(88-97)). The median values of all the scales applied to the participants according to the groups are shown in Table 1 and Figure 1. In the Pearson correlation analysis performed with age and scale scores, it was determined that self-compassion value showed a strong positive correlation with self-esteem ($r=0.594$, $p<0.001$) and an inverse correlation with depression level ($r=-0.541$, $p<0.001$). In anxiety disorder patients, it was found that the strongest correlation of self-compassion was with the depression score negatively ($r=-0.586$, $p<0.001$), and the second strongest correlation was with the level of self-esteem ($r=0.576$, $p<0.001$). In healthy controls, it was observed that the strongest correlation with self-compassion was self-esteem ($r=0.586$, $p<0.001$). Correlation levels in patients with depression are given in Table 2, correlation levels in patients with anxiety disorder are given in Table 3, and correlation levels in healthy controls are given in Table 4.

Table 1. Sociodemographic variables and scales scores of all groups

		Groups			
		Depression	**Anxiety**	**Control**	
		n	**n**	**n**	**p**
Gender	**Female**	76	68	64	0.174
	Male	24	32	36	
Marital status	**Single**	55	44	47	0.283
	Married	45	56	53	
Level of education	**Primary-secondary school**	37	32	6	<0.001*
	High school	34	34	46	
	University	29	34	48	
Employment status	**Student**	19	14	13	0.368
	Employed	35	42	53	
	Housewife	32	31	27	
	Unemployed/Retired	14	13	7	
Age	**Mean±sd**	34.7±11.4	35.1±12.5	31.6±8.9	0.104
Psychological Well-Being Scale	**Median (Q1-Q3)**	34(24-42)	40(34-38)	47(44-50)	<0.001* p_a: <0.001 p_b: <0.001 p_c: <0.001
Self-Compassion Scale		60(48-72)	71(58-84)	93(88-97)	<0.001* p_a: 0.001 p_b: <0.001 p_c: <0.001
Rosenberg Self-Esteem Scale		24(21-28)	28(25-32)	34(31-36)	<0.001* p_a: <0.001 p_b: <0.001 p_c: <0.001
Beck Anxiety Inventory		21(11-32)	26(18-33)	4(2-5)	<0.001* p_a: 0.65 p_b: <0.001 p_c: <0.001
Beck Depression Inventory		30(21-37)	16(10-23)	4(3-6)	<0.001* p_a: <0.001 p_b: <0.001 p_c: <0.001

*: $p<0.01$; p_a: Depression - Anxiety disorders; p_b: Depression - Healthy controls; p_c: Anxiety disorders - Healthy controls

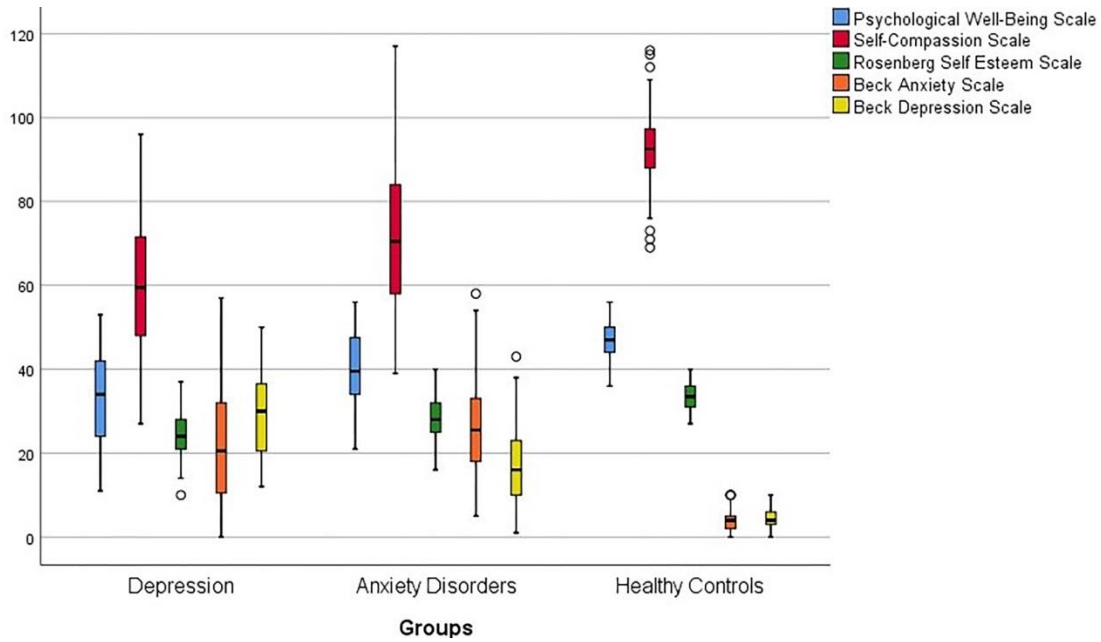

Figure 1. The scales used according to the patient groups and the healthy control group

Table 2. Pearson Correlation between scales scores of depression group

Pearson Correlation		Age	Psychological Well-Being Scale	Self-Compassion Scale	Rosenberg Self-Esteem Scale	Beck Anxiety Inventory	Beck Depression Inventory
Age	**r**	1					
	p						
Psychological Well-Being Scale	**r**	-.027	1				
	p	.787					
Self-Compassion Scale	**r**	.099	.402**	1			
	p	.328	.000				
Rosenberg Self-Esteem Scale	**r**	.151	.572**	.594**	1		
	p	.135	.000	.000			
Beck Anxiety Inventory	**r**	-.040	-.360**	-.422**	-.434**	1	
	p	.694	.000	.000	.000		
Beck Depression Inventory	**r**	.055	-.540**	-.541**	-.491**	.701**	1
	p	.585	.000	.000	.000	.000	

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3. Pearson Correlation between scales scores of anxiety disorders group

Pearson Correlation		Age	Psychological Well-Being Scale	Self-Compassion Scale	Rosenberg Self-Esteem Scale	Beck Anxiety Inventory	Beck Depression Inventory
Age	**r**	1					
	p						
Psychological Well-Being Scale	**r**	.211*	1				
	p	.035					
Self-Compassion Scale	**r**	.198*	.457**	1			
	p	.049	.000				
Rosenberg Self-Esteem Scale	**r**	.147	.566**	.576**	1		
	p	.144	.000	.000			
Beck Anxiety Inventory	**r**	.012	-.443**	-.468**	-.540**	1	
	p	.905	.000	.000	.000		
Beck Depression Inventory	**r**	-.273**	-.530**	-.586**	-.603**	.676**	1
	p	.006	.000	.000	.000	.000	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 4. Pearson Correlation between scales scores of healthy control group

Pearson Correlation		Age	Psychological Well-Being Scale	Self-Compassion Scale	Rosenberg Self-Esteem Scale	Beck Anxiety Inventory	Beck Depression Inventory
Age	**r**	1					
	p						
Psychological Well-Being Scale	**r**	-.018	1				
	p	.858					
Self-Compassion Scale	**r**	.173	.306**	1			
	p	.086	.002				
Rosenberg Self-Esteem Scale	**r**	-.049	.390**	.586**	1		
	p	.626	.000	.000			
Beck Anxiety Inventory	**r**	-.088	-.477**	-.424**	-.351**	1	
	p	.386	.000	.000	.000		
Beck Depression Inventory	**r**	.127	-.329**	-.314**	-.236*	.605**	1
	p	.209	.001	.001	.018	.000	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

According to the findings of this study, which sought to investigate differences in self-compassion levels in depression and anxiety disorders, as well as the relationship of self-compassion with self-esteem and well-being, self-compassion was lower in both anxiety disorder patients and depressed patients compared to healthy controls. In all groups, it was observed that self-compassion was more strongly correlated with self-esteem than well-being. In addition, in both the depression and anxiety groups, self-compassion was found to be strongly and inversely correlated with the depression score.

The finding that self-compassion was lower in patient groups compared to healthy controls is in line with previous studies (30). The higher levels of self-compassion in patients with anxiety disorders compared to patients with depression suggest that depression has a significant effect on self-compassion. The strong correlation between self-compassion and depression levels in the patient groups in the correlation analysis method further supported this conclusion. In studies investigating the relationship between depression and self-compassion, it has been suggested that individuals with high self-compassion reduce self-criticism and negative self-evaluation (31). In cases where self-criticism and negative self-evaluation decrease, individuals' self-esteem is expected to increase. Considering that self-esteem is also lower in depression patients among the findings of the study, it would not be wrong to say that self-compassion is more effective in depression patients than in anxiety patients. Many studies in the literature support low self-esteem in patients with depression (16,32,33).

Another interesting finding from our research is that depressive symptoms in anxiety disorder patients predict self-compassion more than anxiety. While anxiety symptoms are more prominent in anxiety disorder patients, the fact that accompanying depressive symptoms are more related to self-compassion is significant in terms of the individual's emotional regulation ability. There is difficulty in emotional regulation in both depression and anxiety disorders. It is known that depressed patients, in particular, avoid negative emotions through rumination, self-blame, and suppression methods (34,35). On the other hand, depressive complaints accompanying anxiety disorders increase the difficulties of emotional regulation (36). In this regard, the stronger correlation between the self-compassion and depression score in patients with anxiety disorder is consistent with the literature. In other words, depressive symptoms added to anxiety disorders will cause a decrease in self-compassion stronger than anxiety symptoms. Alternatively, anxiety disorder patients with low self-compassion will exhibit more depressive symptoms.

Self-compassion can reduce the negative emotional impact of mistakes and failures, making it easier for an individual to achieve one’s life goals. In other words, balancing one's positive and negative experiences leads to psychological well-being (37). According to our findings, patients with depression had the lowest well-being score, implying that the depressed individual could not achieve well-being due to the effect of self-compassion. However, since our study is a cross-sectional study, it is difficult to determine which is the cause and which is the result between depressive symptoms, self-compassion and psychological well-being. The individual may show less compassion for himself with the effect of the depressive symptoms he experiences, his well-being may decrease as a result, or he may show a tendency to depressive symptoms due to low self-compassion and psychological well-being levels.

Our study has limitations in that it is a cross-sectional study, the clinical scales used in the study are self-rating scales, and the sub-headings of anxiety disorders are not addressed. Even so, the study's large sample size and the fact that it is one of the few studies comparing depression and anxiety disorder patients in terms of self-compassion are valuable aspects of our research.

CONCLUSION

The level of self-compassion decreases in depression and anxiety disorders. This is associated with self-esteem and well-being. As a result, it has been determined that it is critical to bring self-compassion to the forefront of the strong correlations it demonstrates with psychological well-being and self-esteem in depression and anxiety disorders accompanied by depressive symptoms. Cohort follow-up studies with the addition of psychotherapeutic interventions for self-compassion to the treatment are expected to lighten the topic.

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RESEARCH ARTICLE

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The Effects of Pathophysiological Changes in Type-2 Diabetic Patients on Thyroid Dysfunction and Nodular Goiter Development in Turkey

ABSTRACT

Objective: The prevalence of thyroid dysfunction is higher in patients with diabetes, and its diagnosis couldenable a beter diabeticmanagement. The purpose of the present study is to examine the impact of pathophysiological changes in patients with Type-2 diabetes on the frequency of thyroid dysfunction, thyroid autoimmunity, thyroid nodule, and thyroid cancer in Turkey.

Method: The study was conducted on a total of 3.276 patients with Type-2 diabetes who underwent thyroid tests and thyroid ultrasonography (US). The demographic characteristics, biochemical and hormonal values, thyroid US reports, and histopathologic reports were collected from electronic records of the patients.

Results: Thyroid autoimmunity positive TPOab 15.9% (n = 524) and/orpositive TGab 9.9% (n = 327), the rate of positivity of both antibodies (TPOab + TGab) in the same patient, and total thyroid autoimmunity was found to be 32.57% (n=1067) in Type-2 diabeticpatients. Thyroid dysfunction wasdetected in 18.3% (n = 602) of these patients. The distribution of thyroid dysfunction was 9.09% (n = 298) subclinical hypothyroidism, 4.1% (n = 135) clinical hypothyroidism, 3.1% (n = 102) subclinical hyperthyroidism, 2.0% (n = 67) clinical hyperthyroidism. Also, 67.9% (n = 2225) thyroid nodules, and 5% (n = 164) thyroid cancer cases were detected.

Conclusion: Thyroid dysfunction was foundincreased in patients with Type-2 diabetes at significant levels.

Keywords: Type-2 Diabetes, Thyroid Dysfunction, Nodular Goiter, Thyroid Autoimmunity.

Türkiye'de Tip-2 Diyabetli Hastalarda Patofizyolojik Değişikliklerin Tiroid Disfonksiyonu Ve Nodüler Guatr Gelişimi Üzerine Etkileri

ÖZET

Amaç: Bu çalışmanın amacı, Türkiye'de Tip 2 diyabetli hastalardaki patofizyolojik değişikliklerin tiroid disfonksiyonu, tiroid otoimmünitesi, tiroid nodülü ve tiroid kanseri sıklığına etkisini incelemektir.

Gereç ve Yöntem: Çalışma, tiroid testleri ve tiroid ultrasonografisi (US) yapılan toplam 3.276 Tip-2 diyabetli hasta üzerinde yapıldı. Demografik özellikler, biyokimyasal ve hormonal değerler, tiroid US raporları ve histopatoloji raporları hastaların elektronik kayıtlarından toplandı.

Bulgular: Tiroid otoimmünite pozitif TPOab %15.9 (n = 524) ve/veya pozitif TGab %9.9 (n=327), aynı hastada her iki antikorun (TPOab + TGab) pozitiflik oranı ve total tiroid otoimmünitesi Tip-2 diyabetli hastalarda %32,57 (n=1067) olabilir. Bu hastaların %18,3'ünde (n=602) tiroid disfonksiyonu saptandı. Tiroid disfonksiyonu dağılımı %9.09 (n=298) subklinik hipotiroidi, %4.1 (n=135) klinik hipotiroidi, %3.1 (n=102) subklinik hipertiroidi, %2.0 (n=67) klinik hipertiroidi idi. Ayrıca %67.9 (n=2225) tiroid nodülü ve %5 (n=164) tiroid kanseri tespit edildi.

Sonuç: Tip-2 diyabetli hastalarda tiroid disfonksiyonunun anlamlı düzeyde arttığı bulundu.

Anahtar Kelimeler: Tip-2 Diyabet, Tiroid Disfonksiyonu, Nodüler Guatr, Tiroid Otoimmünitesi.

INTRODUCTION

Thyroid hormones are necessary for the carbohydrate metabolism (1). The effect of the secretion of insulin in excessive thyroid hormone may affect many aspects of carbohydrate metabolism over clearance. Thyroid hormone deficiency may prevent insulin secretion and metabolism, resulting in insulin resistance (2,3). Hyperglycemia may develop in dysfunction of the thyroid (4).

The thyroid dysfunction prevalence is higher in diabetic patients. Some organizations recommend that thyroid tests are performed for diabetic patients (5). Recognizing the presence of thyroid dysfunction in Type-2 diabetes patients results in a good management of diabetes (6). Also, some previous studies reported that thyroid nodule incidence is significantly higher in Type 2 diabetes patients compared to healthy subjects (7). In the present study, the purpose was to examine whether the pathophysiological changes in Type-2 diabetic patients hadimpacts on the frequency of thyroid dysfunction, thyroid autoimmunity, thyroid nodule, and thyroid cancer in our country, where a moderate level of iodine deficiency has been encountered.

MATERIAL AND METHODS

This study was performed on a total of 3.276 patients with Type-2 diabetes, followed up in the endocrinology and general surgery clinic between 2015-2021, and underwent thyroid tests and thyroid ultrasonography (US). The demographic characteristics, biochemical and hormonal values, thyroid US reports, and histopathology reports after their thyroid surgeries were collected from patient files and electronic records. The study was conducted with the approval of the Clinical Research Ethics Committee at Medicana International Samsun Hospital in line with the ethical rules (Date 16th April 2020-Issue Number: 07; (1):7123).

Pregnant women, those who were receiving antithyroid drugs or thyroid hormones, those using drugs that disrupt thyroid functions, or who had a radiation history to the head and neck area or who underwent thyroidectomy were excluded from the study. Type-2 diabetic patients over 18 were included in the study.

The Thyroid Function Evaluation Index was calculated by measuring free-T4 (cut-off level 4.6-11.2 µg / dL), free T3 (cut-off level 2.5-5 pg / mL), serum thyroid stimulating hormone (TSH, cut-off level 0.45-4.12 mU / L), thyroid peroxidase antibody (TPOab, cut-off level 0-35 IU / mL), and thyroglobulin antibody (TGab, cut-off level 0-40 IU / ml) levels with the immunochemiluminescent tests in an automated analyzer (Mindray CL-900i).The thyroid nodules and parenchyma of the participants were evaluated by the same endocrinologist by using the same US device (The *Philips* Affinity *70 ultrasound;* Philips North America Corporation 3000 Minuteman Road M/S 109 Andover, MA 01810, USA). The glycated hemoglobin Type A1c (HbA1c cut-off level ≥6.5%) and fasting plasma glucose (cut-off level 70-110 mg / dl) values of Type 2 diabetic patients were used in the study.

Statistical Analyses: The data were expressed as Mean ± Standard Deviation (SD). The categorical data were compared with the Chi-Square Test, and the continuous variables were compared with the Unpaired Student t-test. The One-way ANOVA and Multiple ***t***-tests were done to compare the biochemical and hormonal values. The Independent Samples ***t***-test was used to compare the differences in clinical characteristics and thyroid nodules. Statistically significant level was taken as $p < 0.05$. The data were analyzed with the SPSS software (Statistical Package for the Social Sciences, version 22.0, Chicago).

RESULTS

The data of 3.276 (1965 females, 1311 males) patients were examined for the study. The mean age of the patients was 56.8 ± 13.5 years. The demographic characteristics of the patients, their biochemical, hormonal values, thyroid nodules in thyroid US reports, and thyroid cancer data in histopathological reports after thyroid surgeries are given in Table 1. Also, it is seen in Table 1 that thyroid autoimmunity positive TPOab 15.9% (n = 524) and / or positive TGab 9.9% (n = 327), the rate of positivity of both antibodies (TPOab + TGab) in the same patient, and total thyroid autoimmunity was found to be 32.57% (n=1067) in Type-2 diabetic patients.

Table 1. Demographicandclinicalcharacteristics

Parameters	Type-2 Diabetes	P value
Age, years (mean ± SD	56.8±13.5	
Female gender, n (%)	1965 (%59.9)	
HbA1c,%, median (IQR)	8.1 (6.2–9.5)	**0.001**
Fasting blood glucose,mg/dL, (Mean ± SD)	185.7±24.6 (115-253)	**0.001**
Thyroid dysfunction, n (%)	602 (%18.3)	**0.001**
Thyroid autoimmunity, n (%)	1067 (%32.57)	**0.001**
Positive TPOAb, n (%)	524 (%15.9)	**0.001**
Positive TGAb, n (%)	327 (%9.9)	**0.001**
Positive TPOAb + TGAb n (%)*	216 (%6.5)	0.075
Thyroid nodule, n (%)	2225 (%67.9)	**0.001**
Thyroid cancer, n (%)	164 (%5)	0.064

TPOAb; thyroid peroxidase antibodies, TGAb; thyroglobulin antibodies, HbA1c; glycated hemoglobin, *Positive TPOAb + TGAb; patient with positivity of bothautoantibody tests

All of the 3.276 patients who were included in the study were diagnosed with Type-2 diabetes. Thyroid dysfunction was detected in 18.3% (n = 602) of these patients. The distribution of thyroid dysfunction was 9.09% (n = 298) subclinical hypothyroidism, 4.1% (n = 135) clinical hypothyroidism, 3.1% (n = 102) subclinical hyperthyroidism, 2.0% (n = 67) clinical hyperthyroidism. Also, 67.9% (n = 2225) thyroid nodules, and 5% (n = 164) thyroid cancer were detected. The thyroid dysfunctions, nodules, cancers, and thyroid hormonal values are shown in Table 2.

Table 2. Thyroid diseases and thyroid hormonal values in Tip-2 Diabeticpatients

Thyroid diseases	% (n)	P value	TSH	sT4	sT3
Clinical hyperthyroidism	%2.0 (n=67)	0.031	0.01	13.5±2.1	8.1±2.4
Subclinical hyperthyroidism	%3.1 (n=102)	0.025	0.12±0.2	9.4±1.1	4.1±0.8
Clinical hypothyroidism	%4.1 (n=135)	0.011	64.5±9.3	3.9±1.1	3.2±0.4
Subclinical hypothyroidism	%9.09 (n=298)	0.001	8.4±2.5	6.3±1.4	2.9±0.3
Thyroid nodüle	%67.9 (n=2225)	0.001	2.6±0.2	8.7±1.3	3.7±0.6
Thyroid cancer	%5 (n=164)	0.001	2.8±0.3	9.3±1.5	3.4±0.5

TSH; *Thyroid Stimulating Hormone*

DISCUSSION

The present study revealed that the thyroid dysfunction prevalence was 18.3% in Type-2 diabetespatients. Different reports were published in the past showing the relation between thyroid dysfunction and diabetes. The reported thyroid dysfunction prevalence ranged between 2.2% and 16% in diabetic patients (8,9,10). It was shown that thyroid dysfunction was between ~6.6%-13.7% in non-diabetic patients (11,12). Previous studies did not investigate the relation between Type 2 diabetes and thyroid disorders sufficiently. Generally, studies were conducted between Type 1 diabetes and thyroid (13). In the light of the findings of previous studies, it was reported that the thyroid dysfunction prevalence in patients with Type 2 diabetes was higher compared to the general population. The present study showed that the incidence rates of subclinical hypothyroidism, thyroid nodules, and autoimmune thyroiditis were significantly higher in Type-2 DM patients than in the normal population.

It was reported in previous studies that subclinical hypothyroidism in Type-2 diabetic patients was the most common thyroid dysfunction (4.8%-9.3%) (14,15). In the present study, it was found that the frequency of subclinical hypothyroidism was 9.0% as the most common thyroid dysfunction. This finding is supported by other studies that reported that subclinical hypothyroidism in Type-2 diabetic patients was the most common subtype of thyroid dysfunction. In the NHANES III study, the subclinical hypothyroidism prevalence was reported to be 5.8 % in women and 3.4 % in men (16). It was found in the present study that the subclinical hypothyroidism prevalence was 1.5-2.6 times higher in Type-2 diabetic patients compared to nondiabetic patients. Many studies showed that the clinical hypothyroidism prevalence varied between 0.2% and 4.8% (17). It was also shown that clinical hypothyroidism was detected at a rate of 1.9% in Type-2 diabetic patients (12). In the present study, it was found 4.1%.

Previous studies showed that the frequency of clinical and subclinical hyperthyroidism varied between 0.5% and 3% (16). Subclinical hyperthyroidism was found varied between 1% and 1.6% in patients with Type-2 diabetes(18,19). The presentstudy disclosed that the frequency of subclinical hyperthyroidism was 3.1%. Previous studies also showed that the clinical hyperthyroidism prevalence was 0.5%-1.5% in Type-2 diabetespatients (20,21). This study detected the frequency of clinical hyperthyroidism as 2.0%. The prevalence of hyperthyroidism is generally less in healthy non-diabetic individuals and Type-2 diabetic patients.

It was shown that thyroid autoimmunity was higher in Type-2 diabetic patients at significant levels. It was found in previous studies that 13%-14.7% positive TPOab, 1.7% positive TGAb, and 5.0% positivity for both autoantibodies (22,23). There are a limited number of studies comparing the thyroid autoantibodies prevalence in Type-2 diabetic patients. In the present study, it was found that 15.9% positive TPOab, 9.9% positive TGab, 6.5% both autoantibody positivity TPOab + TGab, and thyroid autoimmunity was 32.57%. In this context, it is possible to speculate that thyroid autoimmunity is higher in Type-2 diabetic patients than in the normal population.

In a meta-analysis of 9 studies conducted on the thyroid nodules prevalence in Type-2 diabetic patients, the prevalence was shown to be 60%, 50% in pre-diabetics, and 43% in non-diabetic population (24). The thyroid nodules prevalence in the general population was reported as 32.4% in China, 34.7% in France, 23.4% in Germany, and 17.0% in Brazil (25). In the present study, the thyroid nodules frequency was 67.9% in patients with Type-2 diabetes. It is very likely that diabetic patients develop thyroid nodules. A meta-analysis that included a 5-center prospective cohort study conducted in the USA and a retrospective cohort study conducted in Israel did not report any significant associations between diabetes and

thyroid cancer (26,27). The present study found that the incidence of thyroid cancer was 5% in Type-2 diabetic patients. In this respect, it is possible to speculate that although the thyroid nodules frequency increases in Type-2 diabetic patients, cancer incidence does not.

CONCLUSION

The results in the present study showed that thyroid dysfunction increased in patients with Type-2 diabetes at significant levels. Clinical and / or subclinical hypothyroidism has been the most common thyroid dysfunction prevalence detected in Turkey. It was confirmed in the present study that the autoimmune thyroiditis increased in diabetic patients. Moderate levels of iodine deficiency have been detected in Turkey. In this context, it was also found that iodine deficiency and Type-2 Diabetes increased the frequency of thyroid nodules at significant levels. The present study showed that there was no general relation between thyroid cancer and diabetes. When the relation between Type 2 diabetes and thyroid disorders is considered, efforts must be made to screen for thyroid diseases in Type 2 diabetic patients.

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RESEARCH ARTICLE

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Investigation of DMFT Index and Saliva Values in Morbidly Obese and Obese Patients

ABSTRACT

Objective: Obesity is a global chronic disease that affects both developed and developing countries. The purpose of this study was to examine the effect of morbid obesity and obesity on the decayed, missing, and filled teeth (DMFT) index and saliva values.

Method: This study included 50 morbidly obese, 50 obese, and 50 control group patients aged 18–68 years. The DMFT index of the patients was determined through a clinical examination. The saliva kit was used to determine the saliva values [unstimulated saliva flow rate (USFR), stimulated saliva flow rate (SSFR), saliva viscosity (SV), saliva pH (SpH), and saliva buffering capacity (SBC)]. The patients were surveyed to learn about their oral health habits, nutritional habits, and socioeconomic status.

Results: No significant difference was found in the number of decayed teeth, filled teeth, USFR, or SV between the groups ($p > 0.05$). The DMFT index, number of missing teeth, SSFR, SpH, and SBC all showed significant differences between the groups ($p < 0.05$). The significant variables associated with DMFT, according to the multiple linear regression model, were the frequency of dental visits ($\beta = 0.365$), age ($\beta = 0.322$), and SSFR ($\beta = -0.256$).

Conclusion: Obese patients have a low saliva rate, low saliva pH, low buffering capacity, high DMFT index, and a high number of missing teeth.

Keywords: DMFT, Obesity, Salivary, Tooth Decay.

Morbid Obez ve Obez Hastalarda DMFT İndeksi ve Tükürük Değerlerinin İncelenmesi

ÖZET

Amaç: Obezite gelişmiş ve gelişmekte olan ülkeleri etkileyen küresel ve kronik bir hastalıktır. Bu çalışmanın hedefi morbid obezite ve obezitenin çürük, kayıp ve dolgulu dişler (DMFT) indeksi ve tükürük değerleri üzerindeki etkilerini araştırmaktır.

Gereç ve Yöntem: Çalışmaya 18-68 yaş arası 50 morbid obez, 50 obez ve 50 normal kilolu hasta dahil edildi. Hastaların DMFT indeksi klinik muayene ile belirlendi. Tükürük değerleri ise [uyarılmamış tükürük akış hızı(USFR), uyarılmış tükürük akış hızı(SSFR), tükürük viskozitesi(SV), tükürük pH'ı(SpH) ve tükürük tamponlama kapasitesi(SBC)] tükürük kiti yardımıyla belirlendi. Hastalar ağız bakım alışkanlıkları, beslenme alışkanlıkları ve sosyoekonomik durumlarını belirlemek amacıyla ankete tabi tutuldu.

Bulgular: Gruplar arasında çürük diş sayısı, dolgulu diş sayısı, USFR ve SV açısından anlamlı fark görülmedi ($p>0.05$). DMFT indeksi, kayıp diş sayısı, SSFR, SpH ve SBC açısından gruplar arasında anlamlı fark bulundu ($p<0.05$). Çoklu lineer regresyon modeline göre diş hekimine gitme sıklığı (Beta=0,365), yaş (Beta=0,322) ve uyarılmış tükürük akış hızı (Beta=-0,256) DMFT ile ilişkili anlamlı değişkenlerdi.

Sonuç: Obez hastalar düşük tükürük akışı, düşük tükürük pH'ı, düşük tamponlama kapasitesi, yüksek DMFT indeksi ve yüksek kayıp diş sayısına sahiptir.

Anahtar Kelimeler: Diş Çürüğü, DMFT, Obezite, Tükürük.

INTRODUCTION

Obesity is a disease characterized by an increase in body fat mass in comparison with lean body mass when the energy consumed exceeds the energy consumed with food (1). Obesity is one of the most serious health problems faced by both developed and developing countries and is considered one of the 10 riskiest diseases by the World Health Organization (WHO) (2).

The most preferred criterion in obesity classification is body mass index (BMI). BMI is calculated by taking one's weight in kilograms and dividing it by one's height in meters squared (3, 4). Individuals are classified as overweight, obese, and morbidly obese according to their BMI (5). Although obesity can be caused by a number of different factors, it is believed that an increase in calorie consumption and physical inactivity contributes to the development of obesity. Nevertheless, many genetic, environmental, neurological, psychological, and sociocultural factors cause obesity (6).

Obesity is associated with many diseases, such as cardiovascular diseases, hypertension, diabetes, cancer, and metabolic syndrome, as well as social, economic, and psychological problems (7, 8). Obesity negatively affects oral and dental health by causing problems such as dental caries, periodontal problems, tooth erosion, tooth loss, and dry mouth (8).

Carbohydrate-rich eating habits, genetic predisposition, socioeconomic level, lack of oral hygiene, saliva amount, and changes in saliva characteristics are common predisposing factors in the occurrence of obesity and tooth decay (9). Tooth loss may increase with the effects of caries and periodontitis, which increase with obesity (10). Due to dry mouth in obese individuals, the role of saliva in protecting oral and dental health may be negatively affected (11).

The decayed, missing, and filled teeth (DMFT) index, which is defined as the sum of decayed, missing, and filled teeth, is one of the most commonly used index systems in the evaluation of dental caries (12). The use of the DMFT index is recommended by the WHO for the measurement and comparison of dental caries in a community (13). The limitations of the DMFT index are that initial enamel lesions are not included in the classification and that the calculation of the DMFT index differs between individuals and between evaluations of the same person at various times (14).

The relationship between morbid obesity and the DMFT index, unstimulated saliva flow rate (USFR), saliva pH (SpH), saliva buffering capacity (SBC), and saliva viscosity (SV) has been recognized in the literature, but more research is needed. The purpose of this study is to investigate the effects of morbid obesity and obesity on the risk of caries in morbidly obese, obese, and normal-weight patients by measuring the DMFT index, USFR, stimulated saliva flow rate (SSFR), SBC, SpH, and SV. The null hypothesis of this study is that morbid obesity and obesity do not negatively affect the DMFT index and saliva values.

MATERIAL AND METHODS

This study was conducted in the Department of Internal Diseases and the Department of Endocrinology and Metabolism Diseases of Inonu University Turgut Ozal Medical Center Training and Research Hospital between September 2019 and February 2020. The study was approved by the Malatya Clinical Research Ethics Committee on April 24, 2019, with decision number 2019/85.

The calculated power (1- β) was 0.826, considering a type I error (alpha) of 0.05, a sample size of 50 in each group (150 in total), and an effect size of 0.67 for the DMFT index. The study included 50 morbidly obese (41 females and 9 males, aged 18–68 years), 50 obese (41 females and 9 males, aged 19–68 years), and 50 normal-weight (26 females and 24 males, aged 25–53 years) patients. Necessary information was given to these patients in both written and verbal forms. With their permission, signed informed consent forms were obtained, and the study commenced.

Calculation of BMI: The weight and height of the patients who participated in the study were measured with a weight/height scale, and their BMI was calculated by taking their weight in kilograms and dividing it by their height in meters squared. Among the individuals whose BMI was calculated, individuals with a BMI value in the range of 18.5–24.9 were classified as normal weight, those in the range of 30–39.9 as obese, and those with a BMI > 40 as morbidly obese (15).

Calculation of the DMFT Index: The DMFT index of the patients was calculated using the criteria published by the WHO in 1997 (16). Clinical examinations were undertaken by a single dentist under a light source with the help of a mirror and a probe. The DMFT indexes of the individuals obtained through clinical examination were recorded in the patient information form.

Determination of Saliva Values: A ready-made GC Saliva Check Buffer kit was used to evaluate the saliva parameters. The patients were informed that they should not smoke, consume food and drinks, brush their teeth, or use mouthwash within 1 h before the procedure. Saliva collection was performed between 9:00 AM and 11:00 AM.

Determination of the USFR: The patients were asked to swallow all of their saliva to determine the USFR. The saliva accumulated for 5 min was collected in a millimeter graduated measuring cup. The amount of unstimulated saliva was measured according to the milliliter (ml) mark on the container, and the flow rate of unstimulated saliva was calculated in milliliter/minute (ml/min) by dividing the value found by 5.

Determination of SpH and SV: The pH band was maintained for 10 s in the container where a patient's unstimulated saliva flow was collected. The pH measurement was made by comparing the color received by the pH band with the scale included in the package. SV was determined by visually evaluating a patient's unstimulated saliva density.

Determination of SSFR: In determining SSFR, paraffin tablets were chewed by the patients to stimulate salivary secretions. After chewing the paraffin tablet for 30 s, each patient was told to spit into the millimeter-grade saliva collection container. The chewing of a paraffin tablet was continued by repeating the spitting process every 15–20 s for 5 min. The quantity of stimulated saliva shown at the millimeter marking on the cup was measured and calculated in ml/min.

Determination of the SBC: Some saliva was extracted from the container containing the patient's stimulated saliva using a pipette, and a drop was deposited on the triple pad on the saliva tamponade test band. The tape was brought upright to spread the saliva onto the absorbent surface. When the band started to change color within 2 min, points were given using a scale according to the color of each pad. The SBC was determined by calculating the total score.

Evaluation of the Survey Findings: The patients completed the survey shown in Table 4 to determine the information to be used in evaluating their education level, socioeconomic status, oral health habits, and nutritional habits. The questionnaires were completed under the dentist's supervision.

Statistical Evaluation: The research data were statistically analyzed using IBM SPSS for Windows, version 22.0. Data related to the quantitative variables were defined as the arithmetic mean (AO) ± standard deviation and median (minimum–maximum). The definition of the data related to the qualitative variables is indicated by a number (n) and percentage (%). To examine the quantitative data, the Shapiro–Wilk normality test was used. One-way analysis of variance (ANOVA) or the Kruskal–Wallis ANOVA was used to compare more than two groups based on the test results (group number > 2). To compare the groups pairwise, the least significant difference method or the Mann–Whitney U test with Bonferroni correction was used. In the analysis of the qualitative variables, Pearson's chi-square test was used. For the Mann–Whitney U test with Bonferroni correction, general analyses with $p < 0.017$ and $p < 0.05$ were considered statistically significant. Age, gender, BMI, SSFR, USFR, SpH, SBC, educational level, frequency of going to the dentist, oral hygiene equipment, socioeconomic status, dessert consumption, and acidic drink consumption, which could be related to the relationship between the DMFT score and the factors, were modeled using multiple linear regression analysis. The stepwise technique was applied in the variable selection process. In testing the significance of the relevant model and its coefficients, p values < 0.05 were considered significant.

RESULTS

The mean ages of the patients in the morbidly obese, obese, and control groups differed significantly statistically ($p = 0.029$, $p<0.05$). A significant difference was found between the obese and control groups when the groups' ages were compared ($p = 0.008$) but not between the morbidly obese and control groups ($p = 0.298$) or between the morbidly obese and obese groups ($p = 0.106$).

Table 1 shows the anthropometric data and the DMFT index distribution of the groups. A statistically significant difference was found between the groups' average weight, height, and BMI ($p < 0.05$). A significant difference was observed between the morbidly obese and obese groups ($p < 0.001$), between the morbidly obese and control groups ($p < 0.001$), and between the obese and control groups ($p < 0.001$) when the groups' weight and BMI were compared. A significant difference was found in height between the morbidly obese and control groups ($p = 0.006$) but not between the obese and control groups ($p = 0.020$) or between the morbidly obese and obese groups ($p = 0.793$). The control group's average BMI and weight were lower than those of the morbidly obese and obese groups. The control group's average height was higher than that of the morbidly obese group.

Table 1. Anthropometric data and DMFT index distribution of the groups (n=50 in each group)

Variables	Control	Obese	Morbidly Obese	p value*
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Weight	65 (48-82)	92 (71-123)	120.5 (85-170)	**<0.001**
Height	1.68 (1.55-1.82)	1.64 (1.40-1.85)	1.62 (1.44-1.90)	**0.013**
BMI	22.7 (19.03-24.80)	34.8 (30.47-39.19)	44.3 (40.23-54.69)	**<0.001**
D	1.0 (0 – 4)	1.0 (0 – 8)	1.5 (0 – 8)	0.057
M	1.0 (0 – 4)	3.0 (0 – 23)	2.0 (0 – 20)	**0.002**
F	4.0 (0 – 15)	2.0 (0 – 16)	1.0 (0 – 15)	0.375
DMFT	6.0 (0 – 20)	10.0 (0 – 24)	9.0 (0 – 25)	**0.020**

Abbreviations: D, decayed tooth; M, missing tooth; F, filling tooth; DMFT, decayed, missing and filled teeth.
*: Kruskal-Wallis Analysis of Variance

No statistically significant difference was found between the groups in terms of D (decayed tooth) and F (filled tooth) values ($p > 0.05$). A statistically significant difference was found in the M (missing tooth) and DMFT index values between the groups ($p < 0.05$). A significant difference was observed between the obese and control groups when the M values of the groups were examined ($p < 0.001$) but not between the morbidly obese and control groups ($p = 0.032$) or between the morbidly obese and obese groups ($p = 0.232$). A significant difference was found between the obese and control groups when the groups were compared using the DMFT index ($p = 0.006$) but not between the morbidly obese and control groups ($p = 0.078$) or between the morbidly obese and obese groups ($p = 0.351$). When compared with the control group, obese patients had a higher number of missing teeth and a higher DMFT index.

Table 1: Anthropometric data and DMFT index distribution of the groups (n = 50 in each group)

When the endocrinological findings of the groups were compared, a statistically significant difference was found in diabetes diagnosis between the obese and morbidly obese groups ($p < 0.001$). Diabetes was observed in 60% of the obese patients, while no diabetes was observed in 40%. Diabetes was detected in 22% of the morbidly obese patients, while no diabetes was detected in 78%.

Table 2 shows the distribution of the groups' saliva values. A statistically noticeable difference was found between the groups according to the SSFR, SBC, and SpH averages ($p < 0.05$). When the groups were compared according to the SSFR, there was a noticeable difference between the morbidly obese and control groups ($p < 0.001$) and between the obese and control groups ($p < 0.001$) but not between the morbidly obese and obese groups ($p = 0.753$). When the groups were compared according to the SBC, a noticeable difference was found between the morbidly obese and control groups ($p = 0.001$) and the obese and control groups ($p = 0.002$) but not between the morbidly obese and obese groups ($p = 0.883$). The control group had a higher SSFR and SBC than the morbidly obese and obese groups. When the groups were compared according to pH distribution, there was a noticeable difference between the obese and control groups ($p = 0.014$) but not between the morbidly obese and control groups ($p = 0.115$) or between the morbidly obese and obese groups ($p = 0.373$). Obese patients had lower SpH than the control group.

Table 2. Saliva values distribution of the groups (n=50 in each group)

Variables	Control	Obese	Morbidly Obese	p value*
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Stimulated Saliva Flow Rate	2.1 (0.4-3.4)	1.2 (0.2-3.0)	1.4 (0.4-3.4)	**<0.001**
Unstimulated Saliva Flow Rate	0.6 (0.20-1.60)	0.4 (0.10-1.04)	0.5 (0.20-1.80)	0.186
Saliva pH	6.8 (6.0-7.4)	6.6 (6.0-7.4)	6.8 (5.8-7.2)	**0.045**
Buffering Capacity	10.0 (3-12)	9.0 (2-12)	8.0 (3-12)	**0.001**

*: Kruskal-Wallis Analysis of Variance

No statistically noticeable difference was found in the groups' mean USFR or SV ($p > 0.05$). The control group had an SV of 8% red (high viscosity), 76% yellow (increased viscosity), and 16% green (normal viscosity); the obese group had 20% red, 56% yellow, and 24% green; and the morbidly obese group had 20% red, 50% yellow, and 30% green.

Table 3 presents the distribution of the groups' survey data. A statistically noticeable difference was found between the morbidly obese, obese, and control groups in terms of gender, educational status, oral hygiene equipment, tooth brushing frequency, frequency of dental floss use, socioeconomic status, sweets consumption, and acidic beverage consumption ($p < 0.05$). No statistically significant difference was observed in their visits to the dentist, the last time they went to the dentist, the reasons for going to the dentist, and their smoking ($p > 0.05$).

Table 4 shows the three significant variables based on the multiple linear regression model results. The variables that contributed the most to the model based on the standardized β coefficient were frequency of going to the dentist ($\beta = 0.365$), age ($\beta = 0.322$), and SSFR ($\beta = -0.256$).

Table 3. Distribution of the groups' survey data (n=50 in each group)

Variables	Control	Obese	Morbidly Obese	p value*
Gender				
Female	26 (52%)	32 (64%)	41 (82%)	**0.006**
Male	24 (48%)	18 (36%)	9 (18%)	
Education level				
Illiterate	0 (0%)	1 (2%)	4 (8%)	
Primary education	2 (4%)	18 (36%)	16 (32%)	
Secondary education	3 (6%)	9 (18%)	6 (12%)	**<0.001**
High school	9 (18%)	15 (30%)	11 (22%)	
University	36 (72%)	7 (14%)	13 (26%)	
Frequency of going to the dentist				
Never go	3 (6%)	3 (6%)	4 (8%)	
Once	1 (2%)	2 (4%)	2 (4%)	0.734
Twice	7 (14%)	2 (4%)	4 (8%)	
Three and more	39 (78%)	43 (86%)	40 (80%)	
When did you last go to the dentist				
Never been	3 (6%)	3 (6%)	4 (8%)	
Less than 3 months ago	15 (30%)	11 (22%)	12 (24%)	
4-6 months ago	8 (16%)	7 (14%)	5 (10%)	
7-12 months ago	12 (24%)	12 (24%)	10 (20%)	0.774
2-4 years ago	8 (16%)	15 (30%)	12 (24%)	
5-7 years ago	3 (6%)	0 (0%)	3 (6%)	
8-10 years ago	1 (2%)	2 (4%)	3 (6%)	
More than 11 years	0 (0%)	0 (0%)	1 (2%)	
Reason for attendance at the dentist				
Never been	3 (6%)	3 (6%)	4 (8%)	
Pain	8 (16%)	9 (18%)	6 (12%)	
Decay	11 (22%)	10 (20%)	11 (22%)	
Extraction	8 (16%)	17 (34%)	12 (24%)	0.139
Periodontal Treatment	11 (22%)	3 (6%)	4 (8%)	
Control	7 (14%)	2 (4%)	4 (8%)	
Other	2 (4%)	6 (12%)	9 (18%)	
Oral hygiene equipment				
None	0 (0%)	1 (2%)	3(6%)	
Toothbrush (TB)	26 (52%)	37 (74%)	32 (64%)	
Floss (F)	0 (0%)	0 (0%)	0 (0%)	
Mouthwash (MW)	0 (0%)	1 (2%)	0 (0%)	**0.002**
TB and F	16 (32%)	3 (6%)	6 (12%)	
TB and MW	5 (10%)	7 (14%)	9 (18%)	
TB, F and MW	3 (6%)	1 (2%)	0 (0%)	
Brushing frequency				
Twice a day or (+)	24 (48%)	9 (18%)	11 (22%)	
Once a day	21 (42%)	18 (36%)	17 (34%)	**0.001**
Rarely	5 (10%)	17 (34%)	18 (36%)	
Never	0 (0%)	6 (12%)	4 (8%)	
Flossing frequency				
Twice a day or (+)	2 (4%)	0 (0%)	0 (0%)	
Once a day	9 (18%)	0 (0%)	1 (2%)	**<0.001**
Rarely	8 (16%)	3 (6%)	5 (10%)	
Never	31 (62%)	47 (94%)	44 (88%)	
Smoking				
Non-smoker	28 (56%)	37 (74%)	31 (62%)	
Light smoker (1-5**)**	7 (14%)	0 (0%)	6 (12%)	0.108
Medium smoker (5-10)	6 (12%)	2 (4%)	5 (10%)	
Heavy smoker (10-15)	9 (18%)	11 (22%)	8 (16%)	
Socioeconomic status				
Low	0 (0%)	8 (16%)	3 (6%)	
Medium	37 (74%)	35 (70%)	40 (80%)	**0.018**
High	13 (26%)	7 (14%)	7 (14%)	
Dessert consumption				
None	1 (2%)	7 (14%)	3 (6%)	
Little	16 (32%)	20 (40%)	18 (36%)	**0.001**
Medium	29 (58%)	12 (24%)	13 (26%)	
Too much	4 (8%)	11 (22%)	16 (32%)	
Asidic drink consumption				
None	14 (28%)	24 (48%)	11 (22%)	
Little	30 (60%)	17 (34%)	23 (46%)	**0.013**
Medium	6 (12%)	6 (12%)	11 (22%)	
Too much	0 (0%)	3 (6%)	5 (10%)	

*: Pearson chi-square test

Table 4. Results of the multiple linear regression analysis modeling

	Unstandardized Coefficients		Standardized Coefficients	t	P	95.0% Confidence Interval for B	
Variables	**B**	**Std. Error**	**Beta**			**Lower Bound**	**Upper Bound**
(Constant)	-5.025	2.674		-1.879	0.062	-10.309	0.259
Age	0.190	0.040	0.322	4.734	**<0.001**	0.111	0.269
Frequency of going to the dentist	2.788	0.507	0.365	5.494	**<0.001**	1.785	3.791
Stimulated saliva flow rate	-2.400	0.634	-0.256	-3.784	**<0.001**	-3.654	-1.146

*: Multiple Linear Regression Analysis; p<0.05.

DISCUSSION

Obesity is a systemic disease with an increasing prevalence and a higher risk of morbidity and mortality (17). Multifactorial factors, such as genetics, dietary habits, age, gender, socioeconomic level, and physical activity, are involved in the etiology of obesity (18). Obesity causes health problems, such as type 2 diabetes mellitus, hypertension, hyperlipidemia, cardiovascular diseases, metabolic syndrome, cancer, infertility, and respiratory system diseases (19). It is also associated with tooth decay (20), periodontal diseases (21, 22), dental erosion (23, 24), and xerostomia (25).

In the data analysis, the number of lost teeth and the DMFT index were higher in the obese group than in the control group, while SSFR, SpH, and SBC were lower in the obese group than in the control group. Thus, the study's null hypothesis was rejected.

Various findings have been obtained in studies looking into the connection between the number of decayed teeth and the DMFT index and obesity in the literature. Isaksson et al. (26) found that obese individuals had a noticeably higher prevalence of caries than normal-weight individuals in their study, which included 494 individuals aged 20 years. Similar to our study, Östberg et al. (27) found no link between the number of carious lesions and obesity in their study, in which 999 women from Gothenburg, Sweden, participated and clinical and radiographic examinations were carried out together.

In the present study, there was no noticeable distinction in the number of decayed teeth between the obese, morbidly obese, and control groups. One of the reasons for this is that no noticeable distinction was found in the USFR values of the groups.

In their study involving 41 obese and 41 normal-weight adolescents aged 12–18 years, Bailleul-Forestier et al. (28) found that the DMFT index of obese adolescents was noticeably higher than that of normal-weight adolescents. In their study of 70 obese and 70 normal-weight women, Şimsek et al. (29) found that the USFR was noticeably lower in obese women than in normal-weight women. Moreover, obese women had significantly higher DMFT and DMFS values than normal-weight women. Unlike our study, Yetkiner et al. (30) found no noticeable differences in BMI and DMFT scores between groups in their study of 527 children classified as underweight, normal, overweight, or obese. Our results demonstrated that the DMFT index was significantly higher in obese individuals than in the control group. Furthermore, the increased number of missing teeth in obese individuals compared with the control group produced a significantly higher DMFT index in obese individuals than in the control group.

Different results were obtained from studies investigating the relationship between SSFR and USFR and obesity. In their study examining the USFR and SSFR of 1,427 dental patients, Flink et al. (31) found that hyposalivation was associated with disease and high BMI (BMI > 25) in young adults and with drugs used in those aged 50 and above. Fenoll-Palomares et al. (32) revealed that obesity, smoking, and alcohol consumption did not affect saliva parameters such as USFR, SpH, and SBC in their study involving 159 healthy volunteers.

The current study found that the SSFR values were significantly greater in the control group than in the morbidly obese and obese groups. This finding confirms that obesity increases the tendency for the occurrence of xerostomia. Moreover, the control group had a significantly higher SpH than the obese group. The SBC values were significantly higher in the control group than in the obese and morbidly obese groups. In this study, a high SpH and the protective effect of SBC were two of the reasons why the DMFT index was significantly lower in the control group than in the obese group.

Among the studies examining the connection between obesity and diabetes, Astrup et al. (33) suggested that the term "diabesity" should be adopted, stating that type 2 diabetes is related to obesity and is primarily caused by obesity. In their study on 195,005 adults over the age of 18 in the United States in 2001, Mokdad et al. (34) examined the connection between obesity and diabetes and found that the prevalence of diabetes increased by 7.3%, with an obesity prevalence rate of 5.6%.

In our study, diabetes was observed in 41% of both groups, 60% of obese individuals, and 22% of morbidly obese individuals. Diabetes causes oral complications, such as hyposalivation, xerostomia, caries, and periodontal diseases (35, 36). As the source of the shortage of statistical importance between the two groups in terms of the number of missing teeth, DMFT index, SSFR, SpH, and SBC,

which are expected to have a worse outcome in morbidly obese patients than in obese patients in proportion to their BMI, the known negative effects of diabetes, which are observed more in obese individuals, on oral and dental health can be shown.

In their study involving 100 morbidly obese and 50 normal-weight individuals, Yamashita et al. (37) found that, consistent with our findings, SSFR and SpH were noticeably lower in obese patients than in the control group. Moreover, they discovered that obese patients had a noticeably higher risk of diabetes and a lower socioeconomic status than the control group.

Although there was an obvious difference in the socioeconomic levels of the participants in our study, no noticeable difference was found in the individuals' status in terms of going to the dentist, the last time they went to the dentist, and the reasons for going to the dentist. These findings show that the difference in socioeconomic level does not have a noticeable difference in the status of going to the dentist, the time, and the reason for going to the dentist. The fear of dentists, which is still common in today's society, and the lack of sufficient awareness in our country about going to the dentist may also be considered factors.

Unlike our findings, Forslund et al. (38) found no significant relationship between education level and obesity in their study of middle-aged, normal-weight, obese, and extremely obese women. Nevertheless, they discovered a noticeable difference in the number of teeth and daily energy intake in normal-weight, obese, and extremely obese women.

The limitations of the study are the small sample size of 50 for each group, the difference in the age variable between the groups, and the inability to completely eliminate systemic diseases from the obese and morbidly obese groups.

CONCLUSION

The findings showed that the DMFT index and the number of lost teeth increased, whereas SSFR, SpH, and SBC decreased in obese individuals. Therefore, obesity has negative effects on oral and dental health. Raising the awareness of obese individuals about oral and dental care and gaining the habit of going to the dentist regularly for obese individuals can contribute to their oral and dental health.

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RESEARCH ARTICLE

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Relationship of Vitamin B12 and Vitamin D with IL-4, IL-10, TNF Beta in Obese Patients

ABSTRACT

Objective: We aimed to investigate the relationship of vitamin B12 and vitamin D with IL-4, IL-10 and TNF-Beta in obese patients.

Method: Serum IL-4, IL-10 and TNF-Beta levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle.

Results: The IL-10 level was found to be significantly lower in the low vitamin D group (p= 0.039). When vitamin B12 normal, vitamin B12 low and control groups were compared, a statistical difference was found between the groups in terms of IL-10 (p=0.002). As a result of post hoc analysis, the IL-10 level was found to be significantly lower in the vitamin B12 low group than in the vitamin B12 normal group (0.04). At the same time, vitamin B12 was statistically higher in the normal group (obesity positive) than in the control group (non-obese vit B12 normal) (p=0.001). A positive correlation was found between vitamin B12 and IL-10 (r= 0.203 p= 0.058).

Conclusion: It has been shown that low levels of vitamin D and vitamin B12 in obese patients cause low levels of IL-10. It was also found that obesity caused an increase in IL-10 levels. No relationship was found between IL-4 and TNF-Beta and vitamin D and vitamin B12. Longer follow-up and studies in larger case populations are needed to better understand the effects of vitamin B12 and vitamin D on IL-4, IL-10 and TNF-Beta levels in obese patients.

Keywords: IL-10, IL-4, Obesity, TNF-Beta, Vitamin B12, Vitamin D.

Obez Hastalarda Vitamin B12 ve Vitamin D' nin IL-4, IL-10, TNF-Beta ile İlişkisi

ÖZET

Amaç: Obez hastalarda vitamin B12 ve vitamin D nin IL-10, IL-4, TNF-Beta ile ilişkisini incelemeyi amaçladık.

Gereç ve Yöntem: Serum IL-10, IL-4 ve TNF-Beta seviyeleri ELISA (Enzyme Linked Immunosorbent Assay) prensibine dayalı kitler kullanılarak ölçülmüştür.

Bulgular: IL-10 düzeyi, vitamin D düşük olan grupta anlamlı olarak daha düşük bulunmuştur (p= 0.039). Vitamin B12 normal, vitamin B12 düşük ve kontrol grupları karşılaştırıldığında IL-10 açısından gruplar arasında istatistiksel düzeyde fark bulunmuştur (p=0.002). Post hoc analizi sonucunda IL-10 düzeyi, vitamin B12 düşük grubunda vitamin B12 normal grubuna göre anlamlı olarak daha düşük olarak tespit edilmiştir (0.04). Aynı zamanda vitamin B12 normal grupta (obezite pozitif) kontrol grubuna (non-obez vit B12 normal) göre daha yüksek olarak belirlenmiştir (p=0.001). IL-10 ile vitamin B12 ve Vitamin D arasında korelasyon bakıldığında vitamin B12 ile IL-10 arasında pozitif korelasyon tespit edilmiştir (r=0.203 p= 0.058).

Sonuç: Obez hastalarda vitamin D ve vitamin B12 düşük seviyelerinin IL-10 düzeyinde düşüklüğe neden olduğu gösterilmiştir. Ayrıca obezitenin de IL-10 seviyesinde artışa neden olduğu belirlenmiştir. IL-4 ve TNF-Beta ile vitamin D ve vitamin B12 arasında ilişki bulunamamıştır. Obez hastalarda vitamin B12 ve vitamin D nin IL-4, IL-10, TNF-Beta düzeylerine etkilerini daha iyi görebilmek için daha uzun süreli takip ve daha geniş vaka popülasyonlarında yapılacak çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: IL-4, IL-10 Obezite, TNF-Beta, Vitamin B12, Vitamin D.

INTRODUCTION

Cytokines are small glycoproteins that are made by immune and non-immune cells. They play a key role in causing or stopping inflammation, angiogenesis or angiostasis, tissue damage or repair, and other things (1). Cytokines regulate inflammation, cell growth, tissue healing and systemic response to injury, immune and inflammatory events (2). Cytokines can be classified as proinflammatory [interleukin (IL)-1β, IL-2, IL-6, IL-12, IL-18, tumor necrosis factor-alpha (TNFα), interferon (IFN)-γ]; anti-inflammatory (IL-4, IL-10, IL-17) and regulatory cytokines (TGF-β, IL-27 and IL-6) (3-7).

Vitamin B12 (cobalamin) is a water-soluble vitamin that contains cobalt, cannot be synthesized in the human body and must be taken from outside. It exists in four forms in the body: cobalamin, methylcobalamin, adenosylcobalamin, hydroxocobalamin, and cyanocobalamin (8). Vitamin B12 is an essential micronutrient required for optimal haemopoietic, neurological and cardiometabolic function (9,10). Oztaş et al. found that vitamin B12 level associated with inflammatory markers and stated that can be used as an inflammatory marker (8). In the literature, vitamin B12 has a regulatory effect on inflammation and proinflammatory cytokines increase in vitamin B12 deficiency has been reported (11). As it is known, especially vitamins A, C, D, E, B2, B6 and B12, folic acid, iron, selenium, zinc and glutamine, arginine, taurine and various amino acids such as sulfur-containing amino acids have an immunomodulatory effect (12).

Vitamin D is formed mostly by the skin through exposure to sunlight and very little of it is taken in through diet. It is converted to 25 OH vitamin D in the liver and to the active form of 25-hydroxyvitamin D (1,25 dihydroxy vitamin D) in the kidney (13,14) The classical roles of vitamin D are regulation of calcium and phosphate metabolism and homeostasis, bone metabolism, and cell growth and division (14). Reported in publications that vitamin D increases the production of some anti-inflammatory cytokines and reduces the release of pro-inflammatory cytokines (15). Experimental studies have shown that 1,25-dihydroxyvitamin D (calcitriol) produces immunological activities on the innate and adaptive immune system and endothelial membrane stability. It has been reported in publications that low serum 25-hydroxyvitamin D [25(OH) D] levels are associated with an increased risk of developing immune-related diseases such as psoriasis, type 1 diabetes, multiple sclerosis, and autoimmune diseases (16).

In this study, we aimed to investigate the relationship between vitamin B12 and vitamin D levels, which have been shown in various studies to be related to inflammation and the immune system in the body, and the anti-inflammatory cytokines IL-4, IL-10 and the regulatory cytokine TNF-Beta in obese patients.

MATERIAL AND METHODS

Patient Selection and Study Design: Fifty obese patients and twenty nine control groups who applied to the Ministry of Health-Ordu University Education and Research Hospital Internal Medicine outpatient clinic were included in the study. The control group consisted of non-obese patients with normal vitamin B12. Vitamin D control group could not be established since the patient who was not obese and had a normal vitamin D level meeting the inclusion and exclusion criteria could not be found sufficiently. Patients with chronic liver disease, malignancy, autoimmune disease, acute and chronic infections, patients taking vitamin therapy, using drugs that have an effect on inflammation, and patients with immune deficiency were not included in the study. In addition, only white-skinned individuals were included in the study.

Sociodemographic characteristics, height and weight, last checked vitamin D and vitamin B12 levels, other hemogram and biochemical parameters, CRP and TSH values of the patients were recorded. BMIs were calculated [BMI = weight (kg)/height^2 (m^2)] (17). Venous blood samples were taken from the patients who accepted the study and gave consent.

Anti-inflammatory cytokines IL-4 and IL-10, regulatory cytokine TNF-Beta biomarkers were studied from these blood samples in Ordu University Medical Faculty Medical Biology Laboratory. Obese patients to be evaluated for vitamin B12 were grouped as vitamin B12 normal (≥200) and vitamin B12 low (<200) (18). A control group was also formed from non-obese vitamin B12 normal patients. Patients to be evaluated for vitamin D were divided into groups as vitamin D normal (≥20) and vitamin D low (<20) (19). Vitamin D control group could not be formed because the patient who was not obese, had normal vitamin D level and met the exclusion criteria, could not be found sufficiently.

Laboratory Analyzes: Serum IL-4, IL-10 and TNF-Beta levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle. Serum IL-4, IL-10 and TNF-Beta was measured using the Cloud-Clone Corp ELISA Kit (Cloud-Clone, USA). The protocols of the relevant kits were followed. Absorbance (450 nm) was measured using the Biotek Epoch 2 microplate reader and Gen5 software. The amounts of IL-4, IL-10 and TNF-Beta in the serum were calculated from the standard curve and the results were expressed as pg/ml. The range of detection was 15.6 to 1.000 pg/ml for IL-4 and TNF-Beta, and 7.8-500 pg/ml for IL-10.

Statistics Analysis: The data were tested in terms of normality with Kolmogorov-Smirnov test. Normally distributed data were analyzed with One-Way Anova test and Student's-t test. Non-normally distributed data were compared with Kruskal Wallis test and Mann-Whitney U test. Categorical data were compared using the Chi-Square test. In the chi-square tests, if a cell had an expected frequency below 5, likelihood ratio chi-square value was used instead of Pearson chi-square value. The numeric variables as mean ± SD and median (min-max), the categorical variables as percentage were expressed. Pearson correlation test was used for normally distributed data and Spearman correlation test was used for non-normally distributed data. SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software was used. Results were evaluated at 95% confidence interval and the significance level was $p < 0.05$.

RESULTS

When the vitamin B12 normal group (obese Vitamin B12 normal), vitamin B12 low group (obese vitamin B12 low) and control groups (non-obese vitamin B12 normal) were compared; there was no difference between the groups in terms of gender, smoking or chronic diseases (Table 1).

Table 1. Comparison of gender and chronic diseases of vitamin B12 groups

	Vit B12 normal (n=33)	Vit B12 low (n=17)	Control group (n=29)	P value
Gender (%)				
Male	10 (29.4)	5 (30.3)	12 (41.4)	0.580
Female	23 (70.6)	12 (69.7)	17 (58.6)	
Hypertension (%)	22 (66.7)	11 (64.7)	13 (44.8)	0.183
Thyroid diseases (%)	7 (21.2)	2 (11.8)	9 (31)	0.294
Coronary Artery Disease (%)	2 (6.1)	0	1 (3.4)	0.423
Heart failure (%)	1 (3)	0	0	0.414
Cigarette (%)	10 (17.6)	3 (30.3)	8 (27.6)	0.606

When the hemogram, biochemical parameters, CRP, TSH, HbA1c, IL-4, IL-10 and TNF-Beta were compared, a difference was found between the groups in terms of blood urea nitrogen (BUN) and IL-10 (respectively p= 0.013, p= 0.002). As a result of post hoc analysis, BUN was found to be significantly lower in the low vitamin B12 group than in the control group (p= 0.009). As a result of post hoc analysis, IL-10 was higher in the vitamin B12 normal group than in the control group (p= 0.001). IL-10 was significantly higher in the vitamin B12 normal group than the vitamin B12 low group (0.04). There was no difference between the groups in terms of IL-4, TNF-Beta (Table 2).

Table 2. Comparison of age and laboratory tests of vitamin B12 groups

	Vit B12 normal (n=33)	Vit B12 low (n=17)	Control group (n=29)	P value
Age (years)	51.27±12.31	49.29±11.72	50.72±15.68	0.665
Hemoglobin (g/dl)	13.66±1.65	13.32±1.78	14.14±1.49	0.233
White blood cell (10^3 uL)	7.63±2.17	6.56±1.94	7.28±1.74	0.171
Platelet (10^3 Ul)	252±67	269±59	257±64	0.706
Fasting blood glucose (mg/dl)	157±73.60	142±65.04	138.63±60.80	0.281
Creatinine (mg/dl)	0.78±0.21	0.68±0.14	0.84±0.34	0.161
Blood urea nitrogen (mg/dl)	14.16±4.67	10.56±2.90	15.96±7.85	***0.013***^{b}
Aspartat aminotransferase (IU/l)	24±15.87	19.41±9.57	17.77±6.32	0.341
Alanine aminotransferase (IU/l)	26.89±17.65	21.41±15.01	18.68±10.11	0.126
Total bilirubin (mg/dl)	0.47±0.21	0.49±0.34	0.51±0.21	0.385
CRP (mg/dl)	0.52±1.32	0.50±0.48	0.69±1.18	0.464
Total cholesterol (mg/dl)	205.4±49.6	196.8±39.1	196.20±44.19	0.837
LDL-cholesterol (mg/dl)	118.8±44.6	165.07±21.6	117.2±38.24	0.979
HDL-cholesterol (mg/dl)	46.07±10.67	45.31±14.10	49.98±12.9	0.262
Triglyceride (mg/dl)	233.35±148.69	196.94±122.77	179.20±205	0.437
TSH (mU/L)	2.64±1.24	2±1.33	2.04±1.43	0.189
HBA1c	7.63±1.99	6.96±1.73	6.98±1.97	0.124
IL-4 (pg/mL)	25±22.26	22.8±29.69	19.15±18.78	0.160
IL-10 (pg/mL)	9.66±0.99	9.44±1.74	8.96±0.88	***0.002***^{a,c}
TNF-Beta (pg/mL)	20.06±1.63	19.72±2.67	18.65±2.94	0.124

CRP: C Reactive protein. TSH: Thyroid stimulating hormone. HBA1c: Hemoglobin A1c. IL-4: Interleukin 4. IL-10: Interleukin 10. TNF-Beta: Tumor Necrosis Factor Beta

a: There is a difference between the control group and the vit B12 normal group

b: There is a difference between the control group and the vit B12 low group

c: There is a difference between vit B12 normal group and vit B12 low group

When the vitamin D low group and vitamin D normal group were compared; there was no difference between the groups in terms of gender, smoking and chronic diseases (Table 3). When compared in terms of hemogram, biochemical parameters, CRP, TSH, HbA1c, IL-4, IL-10 and TNF-Beta; BUN was higher in the vitamin D normal group (p= 0.011). IL-10 was found to be significantly higher in the vitamin D normal group (p= 0.039). There was no difference between the groups in terms of IL-4, TNF-Beta (Table 4).

Table 3. Comparison of gender and chronic diseases of vitamin D groups

	Vit D normal (n=34)	**Vit D low (n=19)**	**P value**
Gender (%)			
Male	11 (32.4)	5 (26.3)	0.646
Female	23 (67.6)	14 (73.7)	
Hypertension (%)	22 (64.7)	12 (63.2)	0.910
Thyroid diseases (%)	8 (23.5)	2 (10.5)	0.229
Coronary Artery Disease (%)	2 (5.9)	0	0.149
Heart failure (%)	0	1 (5.3)	0.177
Cigarette (%)	11 (32.4)	3 (15.8)	0.177

Table 4. Comparison of age and laboratory tests of vitamin D groups

	Vit D normal (n=34)	**Vit D low (n=19)**	**P value**
Age (years)	50.53±12.09	50.05±11.42	0.889**
Hemoglobin (g/dl)	13.73±1.73	13.1±1.73	0.280**
White blood cell (10^3 uL)	7.14 (3.08-12.6)	6.38 (4.01-11.42)	0.105*
Platelet (10^3 Ul)	262.70±80.79	264.78±59.14	0.922**
Fasting blood glucose (mg/dl)	132 (87-278)	114.5 (90-305)	0.303*
Creatinine (mg/dl)	0.71 (0.51-1.24)	0.65 (0.46-0.94)	0.810*
Blood urea nitrogen (mg/dl)	13.6 (6.9-23.2)	10.25 (7.2-15.8)	***0.011****
Aspartat aminotransferase I(U/l)	19 (11-74)	19 (6-50)	0.543*
Alanine aminotransferase (IU/l)	22 (11-77)	16 (8-64)	0.102*
Total bilirubin (mg/dl)	0.44 (0.23-1.18)	0.42 (0.27-1.69)	0.710*
CRP (mg/dl)	0.3 (0.08-5.8)	0.33 (0.07-3.37)	0.578*
Total cholesterol (mg/dl)	206.73±48.95	199.0±38.48	0.556**
LDL-cholesterol (mg/dl)	109.2 (52.5-231.1)	117.3 (62.5-995.7)	0.783*
HDL-cholesterol (mg/dl)	45.7±10.7	44.4±13.5	0.697**
Triglyceride (mg/dl)	154 (83-646)	167 (77-594)	0.591*
TSH (mU/L)	2.47 (0.79-5.24)	1.72 (0.47-4.88)	0.368*
HBA1c	7.2 (5-10.6)	6.05 (5.6-11)	0.106*
IL-4 (pg/mL)	15.6 (15.6-105)	15.6 (15.6-138.02)	0.226*
IL-10 (pg/mL)	9.35 (8.59-12.34)	8.78 (8.21-15.6)	***0.039****
TNF-Beta (pg/mL)	19.83 (17.44-24.36)	19.02 (17.06-29.05)	0.140*

CRP: C Reactive protein. TSH: Thyroid stimulating hormone. HBA1c: Hemoglobin A1c. IL-4: Interleukin 4. IL-10: Interleukin 10. TNF-Beta: Tumor Necrosis Factor Beta

*Mann-Whitney U test, **Student's-t test

When the correlation between IL-10 and vitamin B12 and vitamin D was examined, a positive correlation was found between vitamin B12 and IL-10 (r=0.203 p= 0.058). No correlation was found between vitamin D and IL-10 (Table 5).

Table 5. Correlation between IL-10 and vitamin B12-vitamin D

	r	**P value**
Vit D	0.203	0.058
Vit B12	0.09	0.411

DISCUSSION

In this study, IL-10 levels were found to be significantly lower in the vitamin D low group in obese patients (p= 0.039). IL-10 level was found to be significantly lower in the vitamin B12 low group than in the vitamin B12 normal group (P=0.04). When the correlation between IL-10 and vitamin B12 and vitamin D was examined, a positive correlation was found between vitamin B12 and IL-10 (r=0.203 p= 0.058). There was no difference between the groups in terms of IL-4 or, TNF-Beta.

Vitamin D functions by binding to the nuclear vitamin D receptor (VDR) and retinoid X receptor to regulate gene transcription. VDR has been identified in many other tissues, including the immune system. Vitamin D is considered to be one of the important regulators of the immune system. It has been shown that all cells of the immune

system, including T cells, express VDR (14). After T cells are activated, they induce 1,25(OH)2 vitamin D by expressing VDR and autocrinely. 1,25(OH)2 vitamin D induces IL-4 while inhibiting the proliferation of mouse and human T cells, producing IFN-γ and IL-17 (14) As part of the adaptive immune system, vitamin D interferes with T lymphocyte proliferation and function. Antigen-activated pluripotent Th0 lymphocytes produce a variety of cytokines, including IL-2, IL-4, IL-10 and interferon γ (IFN-γ). Calcitriol directly inhibits the expression of Th1 cytokines (IL-2, IFN-γ, tumor necrosis factor) and stimulates Th2 cytokines (IL-3, IL-4, IL-5, IL-10) (16). Consistent with the literature, we found the IL-10 level, known as an anti-inflammatory cytokine, to be low in the obese vitamin D low group in this study.

It is mentioned in publications that vitamin B12 deficiency increases inflammation (20,21), since vitamin B12 has anti-inflammatory activity (22). Vitamin B12 deficiency causes hyperhomocysteinemia (HHcy), and high homocysteine is known to cause an inflammatory state (23). In the literature, there is no correlation between vitamin B12 level and IL10. In this study, it was found that IL10, an anti-inflammatory cytokine, was low in obese patients with low vitamin B12. The anti-inflammatory activity of vitamin B12 can be explained by this mechanism other than homocysteine.

Interleukin 4 (IL-4) plays a role in the development and upregulation of Th2 cells, is required for the biosynthesis of IgE (24). A number of inflammatory cells including T helper type 2 (Th2) cells, innate lymphoid cells, mast cells and basophils secrete interleukin (IL-) 4 and IL-13 (25,26). IL-4 may have a profibrotic effect on scleroderma dermal fibroblasts. These cells have IL-4 receptors and when cultured in media containing IL-4, collagen production is significantly increased (27). IL-4 shares biological properties and receptor specificity with the related cytokine IL-13. Clinical trial results suggest that blocking IL-4 and IL-13 may benefit SSc skin fibrosis (28). In this study, no relationship was found between vitamin B12, vitamin D and IL-4.

Increased TNF-Beta expression is associated with the formation of lymphocytic aggregates (29). It has been reported in publications that it is one of the inhibitory cytokines such as IL-10 and tumor necrosis factor (TNF)-Beta. (30). In this study, we could not find a relationship between TNF-Beta and vitamin D or vitamin B12.

CONCLUSION

It has been shown that low vitamin D and vitamin B12 levels in obese patients cause low IL-10 levels. At the same time, it was determined that obesity caused an increase in IL-10 level. No relationship was found between IL-4 and TNF-Beta and vitamin D and vitamin B12.

Ethics Statement: This study was approved by the Ordu University, Faculty of Medicine Institutional Review Board Ethics Committee (date: 07.10.2021 number: 2021/ KAEK 232).

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RESEARCH ARTICLE

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Age- and Sex- Dependent Changes in Serum Levels of TAS, TOS, TLR2, TLR4, HSP60, HSP90, and HMGB1

ABSTRACT

Objective: Cellular and physiological functions may be affected in an age- and sex-specific manner. The aim of this study is to investigate sex- and age-specific differences in the serum levels of Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Oxidative Stress Index (OSI), Toll-Like Receptor 2 (TLR2), Toll-Like Receptor 4 (TLR4), Heat Shock Protein 60 (HSP60), Heat Shock Protein 90 (HSP90), and High Mobility Group Box 1 (HMGB1) as well as to examine the correlation between them.

Method: Four groups of mice, each including seven animals, were used in the present study: young males and females (6 months old); old males and females (24 months old). Blood samples were taken from the heart and serum was used to assay the levels of TLR2, TLR4, HSP60, HSP90, HMGB1, TAS and TOS.

Results: HGMB1, TOS and OSI were higher in old females than in young females (p<0.05). TLR2 and TLR4 levels were higher in young females than in young males; however, HSP60 was lower in young females than in young males (p<0.01). HSP60 was lower in old males than in young males (p<0.05). Positive correlations were present between TLR2, TLR4 and HMGB1 (p=0.001, r=0.096; p=0.012, r=0.867; p=0.002, r=0.935, respectively) as well as between HMGB1 and HSP60 (p=0.049, r=0.756) in young females. A negative correlation was detected between HSP90 and TLR4 in young males (p=0.000, r=-0.982), and between HSP60 and TLR2, OSI in old males (p=0.014, r=-0.856; p=0.042, r=-0.772, respectively).

Conclusion: The results of present study indicated that age and sex may be important factors for serum levels of TLR2, TLR4, HSP60, HSP90, HMGB1 and OSI as well as the correlation between them.

Keywords: Sex, Age, High Mobility Group Box 1, Heat Shock Proteins, Toll-Like Receptors, Oxidative Stress.

TAS, TOS, TLR2, TLR4, HSP60, HSP90 ve HMGB1 Serum Düzeylerinde Yaşa ve Cinsiyete Bağlı Değişiklikler

ÖZET

Amaç: Hücresel ve fizyolojik fonksiyonlar yaşa ve cinsiyete özgü bir şekilde etkilenebilir. Bu çalışmanın amacı, Toplam Antioksidan Durumu (TAS), Toplam Oksidan Durumu (TOS), Oksidatif Stres İndeksi (OSI), Toll-Benzeri Reseptör 2 (TLR2), Toll-Benzeri Reseptör 4 (TLR4), Isı Şok Proteini 60 (HSP60), Isı Şok Proteini 90 (HSP90) ve Yüksek Mobilite Grup Kutusu 1 (HMGB1) serum seviyelerindeki cinsiyete ve yaşa özgü farklılıkları ve aralarındaki korelasyonu incelemektir.

Gereç ve Yöntem: Bu çalışmada her biri yedi hayvan içeren dört fare grubu kullanıldı: genç erkekler ve dişiler (6 aylık); yaşlı erkekler ve dişiler (24 aylık). Kan örnekleri kalpten alındı ve TLR2, TLR4, HSP60, HSP90, HMGB1, TAS ve TOS seviyelerini değerlendirmek için serum kullanıldı.

Bulgular: HGMB1, TOS ve OSI yaşlı dişilerde genç dişilere göre daha yüksekti (p<0,05). TLR2 ve TLR4 seviyeleri genç dişilerde genç erkeklerden daha yüksekti; ancak HSP60 genç dişilerde genç erkeklere göre daha düşüktü (p<0,01). HSP60 yaşlı erkeklerde genç erkeklere göre daha düşüktü (p<0,05). Genç dişilerde TLR2, TLR4 ve HMGB1 arasında (sırasıyla p=0,001, r=0,096; p=0,012, r=0,867; p=0,002, r=0,935) ve HMGB1 ile HSP60 arasında (p=0,049, r=0,756) pozitif korelasyonlar mevcuttu. Genç erkeklerde HSP90 ile TLR4 arasında (p=0,000, r=-0,982), yaşlı erkeklerde HSP60 ile TLR2 ve OSI arasında negatif korelasyon saptandı (sırasıyla p=0,014, r=-0,856; p=0,042, r=-0,772).

Sonuç: Bu çalışmanın sonuçları, TLR2, TLR4, HSP60, HSP90, HMGB1 ve OSI'nin serum düzeyleri ve aralarındaki korelasyon için yaş ve cinsiyetin önemli faktörler olabileceğini göstermiştir.

Anahtar Kelimeler: Cinsiyet, Yaş, Yüksek Mobilite Grup Kutusu 1, Isı Şok Proteinleri, Toll Benzeri Reseptörler, Oksidatif Stres.

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INTRODUCTION

The issue of age- and sex- related differences plays a vital role in our understanding of differences in physiological and pathophysiological mechanisms. In recent years, researchers have shown an increased interest in age- and sex-specific changes in several diseases, including cancer, cardiovascular, metabolic, coronavirus disease, and neural diseases (1-4). To date, however, cellular mechanism(s) underlying age- and sex-related changes in several diseases has not been fully elucidated. In addition, under normal physiological conditions age- and sex- specific variability in serum biomarkers and/or proteins such as oxidative stress markers, High Mobility Group Box 1 (HMGB1), Heat Shock Proteins (HSPs), and Toll-Like Receptors (TLRs) are likely to be present. In fact, it should be stated that little is known regarding differences in them depending on age and sex.

Oxidative stress can be called as a vital signaling factor in various cellular pathways, by which apoptosis or survival is achieved. Oxidative stress is observed when the antioxidant defense system is submerged by oxidants. Excess oxidative stress can alter cellular signaling transduction by which it can take a part in the process of several diseases. Oxidative stress degree has linked with age and sex. It increases with aging (5), and males have higher oxidative stress than age-matched females (6).

HMGB1 and HSPs, which are members of the protein family known as damage-associated molecular pattern molecule (DAMP), are released out of the cell in the presence of intracellular stress such as oxidative stress. In response to oxidative stress, activation and/or release of HMGB1 and HSPs are important to whether a cell goes to apoptosis or survive (7, 8). HMGB1 and HSPs as inducers, sensors, and mediators of stress can mediate their effects via binding to TLRs on the cell membrane (9, 10). For example, HMGB1 and HSPs could induce inflammatory cells to active through TLR2 and TLR4 (11). In addition, according to the intracellular pathway activated in this binding, cell death pathway, inflammation, or cell survival pathways are activated. Of the TLR receptors, TLR2 is generally associated with cell survival or regeneration, while TLR4 is reported to be associated with cell death pathways (12).

With advance age, circulating levels of DAMP become elevated, which is proposed that they are likely to be crucial biomarkers to predict or evaluate the risk for disease (10). However, whether levels of oxidative stress, TLR2, TLR4, HSP60, HSP90, and HMGB1 vary with age and/or sex, and whether there is a correlation between them is unclear under normal physiological processes. Overall, the aim of this study is to explore age- and sex-specific differences in the circulating levels of TAS, TOS, TLR2, TLR4, HSP60, HSP90, and HMGB1.

MATERIAL AND METHODS

Experimental Animals: Experimental protocols were approved by the Local Animal Ethics Committee of Düzce University, Düzce, Türkiye (Approval no: 2021/03/06). Animals were exposed to a 12 h- light/dark cycle, and they were fed with a standard pellet food and water *ad libitium*. Four groups of mice (*M. musculus*), each including seven animals, were used in the present study: young males and females (6 months old); old males and females (24 months old).

Biochemical Analyses

Blood Sample: Experimental animals were weighted and then anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). A blood sample was collected by cardiac puncture and allowed to clot at room temperature for 30 min. The samples were then centrifugated at 4000 rpm for 10 min at +4°C. Samples were stored at -80 until analysis. The serum was used to assay the levels of TLR2, TLR4, HSP60, HSP90, HMGB1, TAS and TOS, following the manufacturer's instructions.

Total Antioxidant Status (TAS): Total antioxidant status (TAS) was measured using commercially available kits (Rel Assay Diagnostic, Ankara, Turkey) with Mindray's BS-300 auto chemistry analyzer according to the manufacturer's instructions. The results were expressed as mmol Trolox equivalent/L (13).

Total Oxidant Status (TOS): TOS levels were measured using commercially available kits (Rel Assay Diagnostic, Ankara, Turkey) with Mindray's BS-300 auto chemistry analyzer according to the manufacturer's instructions. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L) (14).

Oxidative Stress Index (OSI): The ratio of TOS to TAS was accepted as the oxidative stress index (OSI) (15, 16). For calculation, the resulting unit of TAS was converted to μmol/L, and the OSI value was calculated according to the following Formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L) / TAS (μmol Trolox equivalent/L).

Measurement of Circulating TLR2, TLR4, HSP60, HSP90 and HMGB1: TLR2, TLR4, HSP60, HSP90, HMGB1 levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, USA) with Biotek ELx800 microplate reader according to protocols provided by the manufacturer.

Statistical Analyses: Data was analyzed with either the Statistical Package for Social Sciences (SPSS: version 21.0; SPSS Inc., IL, USA)

or GraphPad Prism (Version 9.3.1, La Jolla, CA). Comparisons between groups for the serum levels of TLR2, TLR4, HSP60, HSP90, HMGB1, TAS, TOS and OSI were performed using two-way ANOVA with age, sex as main factors, and age-by-sex interactions. A Bonferroni post-hoc. test was used when the effect of factor(s) and/or interactions on the dependent variables were significant. Relationships between TLR2, TLR4, HSP60, HSP90, HMGB1, TAS, TOS, and OSI in young males and females, and old males and females, separately were analyzed with Pearson's *r* test for correlation. Results were expressed as the mean ± standard deviation (mean ± SD). *p* values less than 0.05 were considered as statistically significant.

RESULTS

TAS, TOS and OSI: Although the overall effects of sex and age on TAS were significant, with no significant sex-by-age interaction effects (Table 1; $p < 0.05$), the individual comparisons between sexes and ages were not. This means that TAS did slightly higher in young females (1.23 ± 0.12 mmol/L) than in young males (1.11 ± 0.19 mmol/L), and it did slightly lower in old males (0.88 ± 0.18 mmol/L) and females (1.07 ± 0.12 mmol/L) when compared to young groups (1.11 ± 0.19 mmol/L and 1.23 ± 0.12 mmol/L, respectively; Figure 1A). There was also a significant age-specific difference in TOS and OSI (Table 1; $p < 0.001$) such that TOS and OSI was significantly higher in old females (20.32 ± 9.97 µmol/L for TOS, and 1.65 ± 0.75 for OSI) than in young females (5.90 ± 2.77 µmol/L for TOS, and 0.50 ± 0.22 for OSI; Figure 1B and C; $p < 0.01$). TOS and OSI tended to be higher in old males than in young males; however, it did not reach statistically a significant level. Taken together, these results indicate that oxidative stress was markedly observed in old females.

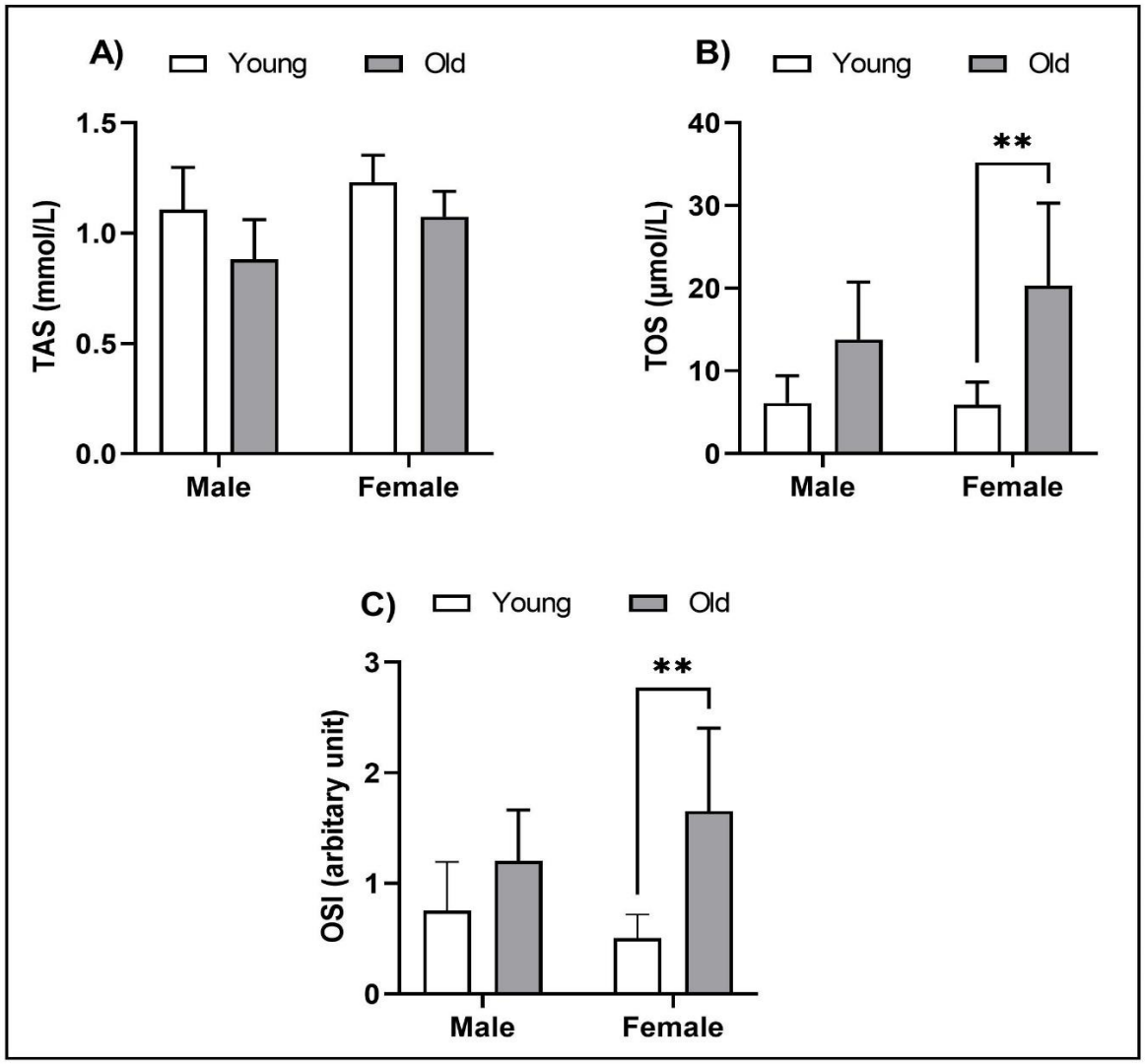

Figure 1. TAS, TOS and OSI in young and old males and females. (Abbreviations: TAS, Total Antioxidant Status; TOS, Total Oxidant Status; OSI, Oxidative Stress Index. Young male, n = 7; old male, n = 7; young female, n = 7; old female, n = 7. Values were expressed as mean ± SD (**p <0.01) and analyzed with two-way ANOVA with a Bonferroni post-hoc. test.)

Table 1 Effects of sex, age and sex-by-age interaction on parameters.

Parameters	*p* value		
	Sex	Age	Sex x Age
TAS	**0.0130**	**0.0035**	0.5511
TOS	0.2454	**0.0001**	0.1471
OSI	0.6094	**0.0003**	0.0784
TLR2	**0.0294**	**0.0035**	0.0762
TLR4	**0.0038**	0.2964	**0.0160**
HSP60	**0.0020**	0.3583	**0.0025**
HSP90	0.4542	0.9946	**0.0091**
HMGB1	0.5269	0.0965	**0.0167**

Abbreviations: TAS, Total Antioxidant Status; TOS, Total Oxidant Status; OSI, Oxidative Stress Index; TLR2, Toll-like Receptor 2; TLR4, Toll-Like Receptor 4; HSP-60, Heat Shock Protein 60; HSP90, Heat Shock Protein 90; HMGB1, High Mobility Group Box 1. Bold values denote significance at $p < 0.05$.

TLR2, TLR4, HSP60, HSP90, and HMGB1: A significant effect of sex and age, with no sex-by-age interaction effect was observed on TLR2 (Table 1; p <0.05), where serum TLR2 level was significantly higher in young females (0.47 ± 0.05 ng/ml) than in young males (0.41 ± 0.04 ng/ml; p <0.05; Figure 2A). In addition, old females had significantly low level of TLR2 (0.39 ± 0.03 ng/ml) when compared to young females (0.47 ± 0.05 ng/ml; p <0.01; Figure 2A). There were a significant effect of sex (p <0.01) and sex-by-age interaction (p <0.05) on TLR4 levels (Table 1), such that TLR4 level was significantly higher in young females (0.54 ± 0.02 ng/ml) than in young males (0.51 ± 0.01 ng/ml; p <0.01; Figure 2B). There was also a significant sex-specific effect on HSP60 level, with a significant sex-by-age interaction effect (Table 1; p <0.01). HSP60 level was significantly lower in young females (0.39 ± 0.10 ng/ml) than in young males (1.08 ± 0.47 ng/ml; p <0.001; Figure 2C). Besides, although there was no significant difference in HSP60 level between young and old females, HPS60 level was significantly lower in old males (0.64 ± 0.17 ng/ml) than in young males (1.08 ± 0.47 ng/ml; p <0.05).

Although the overall effect of sex-by-age interaction on HSP90 was significant (Table 1; p <0.05), post-hoc analyses did not show a significant difference in the individual sex and age groups. This means that HSP90 level was slightly lower in old males (82.90 ± 34.21 pg/ml) than in young males (117.40 ± 31.05 pg/ml); however, it was slightly higher in old females (108.10 ± 35.69 pg/ml) than in young females (73.75 ± 26.74 pg/ml; Figure 2D). In addition, young females had a modest low level of HSP90 when compared to young males.

The overall sex- and age- related differences in serum HMGB1 level were no significant; however, a significant sex-by-age interaction was observed (Table 1; p <0.05), where serum HMGB1 level was significantly higher in old females (45.64 ± 15.07 pg/ml) than young females (30.71 ± 6.77 pg/ml; p <0.05; Figure 2E). By contrast, serum HMGB1 level was slightly lower in old males (34.48 ± 5.60 pg/ml) than in young males (37.41 ± 5.76 pg/ml; p <0.05), with not statistically significance.

Correlation Analysis: Pearson Correlation Analysis was used to determine the relationship between TLR2, TLR4, HSP60, HSP90, HMGB1, TAS, TOS, and OSI. The investigated correlation may be of different size and shape in different subgroups. If the factors such as age and sex were not separately examined in the correlation analysis, a high correlation would be obtained. Therefore, the correlation analysis was performed separately in young males and females, and in old males and females.

In young males, the results of the correlation analysis are set out in Figure 3A. A strong negative (r = -0.982) and significant (p <0.01) correlation were found between TLR4 and HSP90 in young males. This means that while TLR4 level increased, HMGB1 level decreased or vice versa. Besides, there was a strong positive correlation with high significance between TOS and OSI (r =0.940; p <0.01).

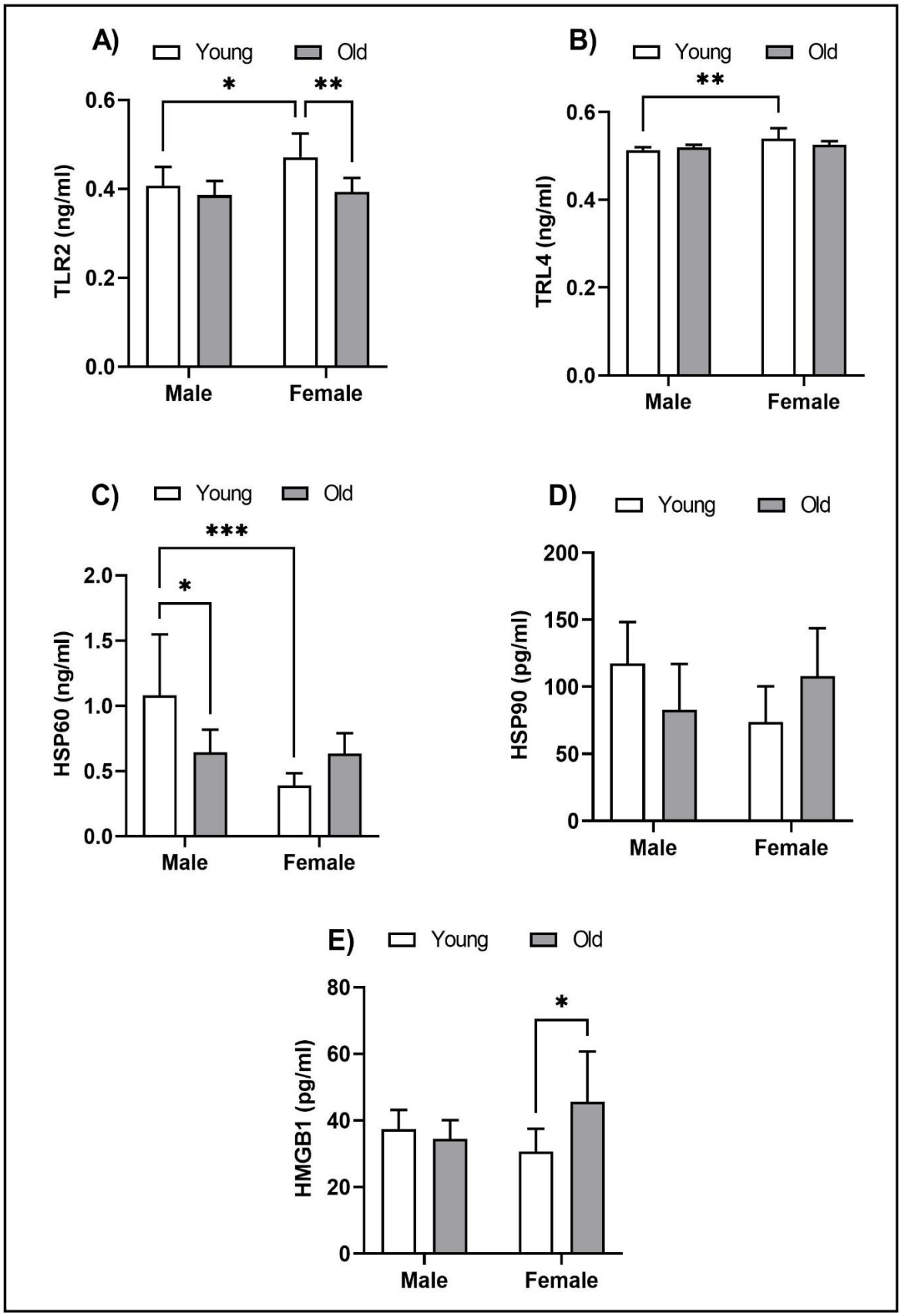

Figure 2. Serum levels of TLR2, TLR4, HSP60, HSP90, HMGB1 in young and old males and females. (Abbreviations: TLR2, Toll-like Receptor 2; TLR4, Toll-Like Receptor 4; HSP60, Heat Shock Protein 60; HSP90, Heat Shock Protein 90; HMGB1, High Mobility Group Box 1. Young male, n = 7; old male, n = 7; young female, n = 7; old female, n = 7. Values were expressed as mean ± SD (*p <0.05; **p <0.01; ***p <0.001) and analyzed with two-way ANOVA with a Bonferroni post-hoc. Test).

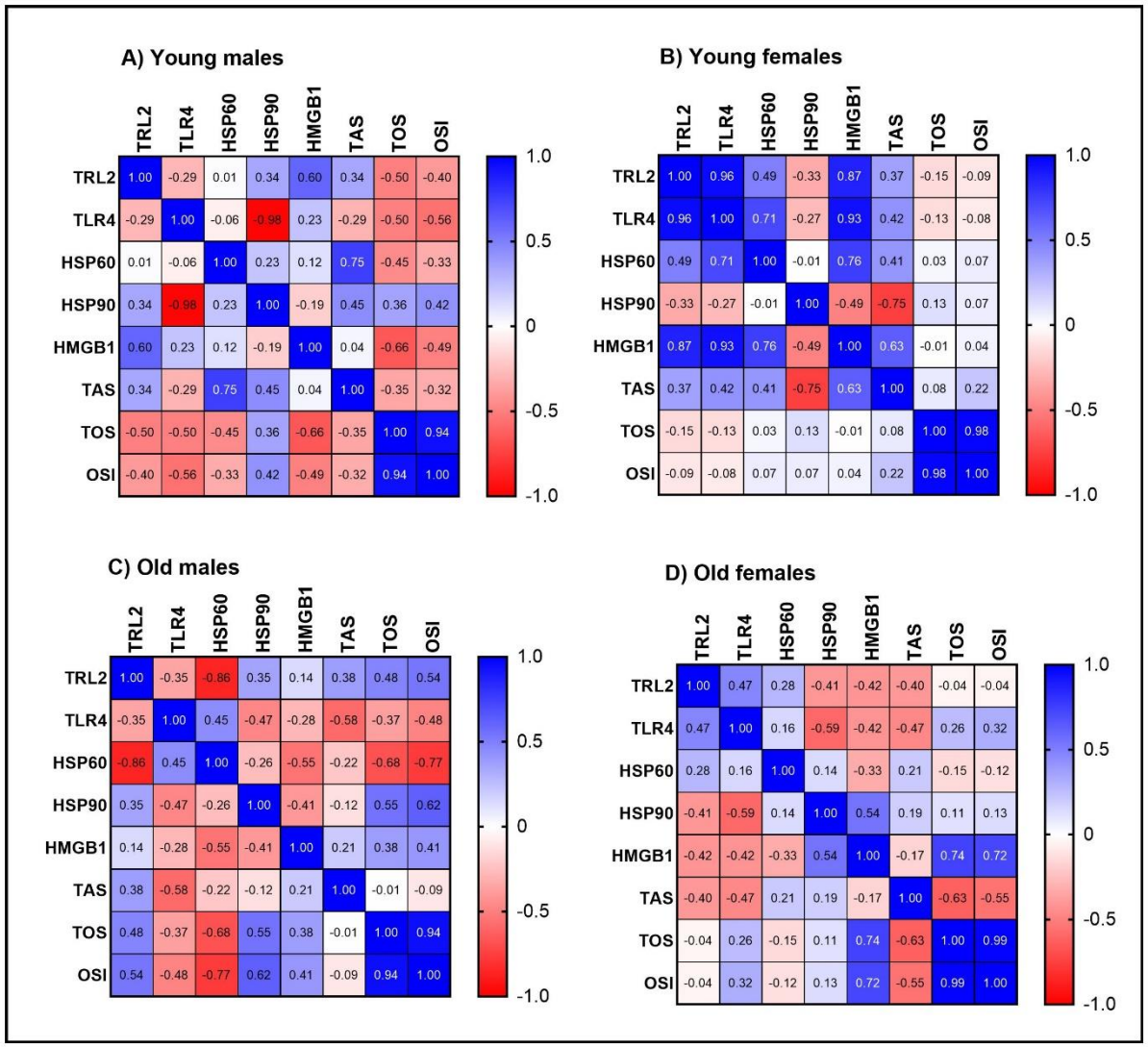

Figure 3. The Pearson correlation matrix of variables. (Abbreviations: TAS, Total Antioxidant Status; TOS, Total Oxidant Status; OSI, Oxidative Stress Index; TLR2, Toll-like Receptor 2; TLR4, Toll-Like Receptor 4; HSP60, Heat Shock Protein 60; HSP90, Heat Shock Protein 90; HMGB1, High Mobility Group Box 1. Young male, n = 7; old male, n = 7; young female, n = 7; old female, n = 7. The color scale bar shows the Pearson r value: the more positive the correlation (closer to 1), the darker the shade of blue; the more negative the correlation (closer to −1), the darker the shade of red).

In young females, the results of the correlation analysis are presented in Figure 3B. The results displayed that a strong positive correlation with high significance was present between TLR2 and TLR4 in young females (r =0.960; p <0.01), which means that TLR2 increased when TLR4 increased or vice versa. Similarly, there were significantly strong correlations between HMGB1 and TLR2 (r =0. 960; p <0.05), TLR4 (r =0.935; p <0.01), and HSP60 (r =0.756; p <0.05). In addition, there was a strong positive correlation with high significance between TOS and OSI (r =0.979; p <0.01), like observed in young males.

In old males, the results of the correlation analysis are shown in Figure 3C. A strong negative (r = 0.856) and significant (p <0.05) correlation was found between TLR2 and HSP60 in old males. This data demonstrates that TLR2 level increased with HSP60 or vice versa. In addition, there was significantly a high correlation between HSP60 and OSI (r =0.772; p <0.05). This finding shows that HSP60 level increased with an increase in OSI. Like data obtained in young males and females, there was significantly a strong correlation between TOS and OSI in old males (r =0.942; p <0.01).

In old females, the results of the correlation analysis are displayed in Figure 3D. A strong correlation with high significance was only observed between TOS and OSI in old females (r =0.986; p <0.01).

DISCUSSSION

To investigate age- and sex-specific changes in possible biomarkers or differences in cellular signaling pathways under physiological and pathophysiological processes is vitally important to

modulate and/or develop new therapeutic approaches. To this end, this study set out to assess changes in circulating levels of TLR2, TLR4, HSP60, HSP90, HMGB1, TAS, and TOS as well as to explore the correlation between them in different sex and age groups. The main findings of the study were (I) TOS, OSI, and HGMB1 were higher in old females than in young females, (II) TLR2 and TLR4 levels were higher in young females than in young males, (III) HSP60 and HSP90 levels were lower in young females than in young males, (IV) HSP60 was lower in old males than in young males, (V) Positive correlations was present between TLR2, TLR4, and HMGB1 as well as between HMGB1 and HSP60 in young females (VI) A negative correlation between HSP90 and TLR4 was found in young males, and also a negative correlation between HSP60 and TLR2, OSI was detected in old males.

Age- and sex-related differences in oxidative stress and the role of such differences in the mechanism underlying the physiological and pathophysiological processes take great attention for a long time (5, 6). In the present study, the findings showed that oxidative stress increased with aging, markedly in the females, evidenced by the fact that TOS and OSI was higher in the old females than in the young females. Contrary to expectations, this study did not find a significant difference TAS, TOS and OSI in the young and old females when compared to age-matched males, which means that oxidative stress is likely to be independent of sex in normal physiologic conditions. It is important to mention that a significant difference in TOS and OSI was observed only between young and old females, rather than in the males, suggesting that the effect of aging on oxidative stress may be more dominate or evident in the females. In accordance with the present results, a previous study indicated that no differences in antioxidant barrier efficacy and oxidative index in rats as well as between age-matched males and females from healthy subjects were obtained, however, oxidative stress in females, but not in males was reported to increase as age increased (17). However, it should be stated that there are some experimental and clinical studies showing a marked significant alternation in oxidative stress between males and females, which are contrary to the results of present study and the mentioned investigation. This conflicting evidence may partly be explained by differences in oxidative stress measurements, methods, markers studies, and samples (18).

HMGB1 is highly conserved protein, which exists from yeast to human. It has been known that circulating level of HMGB1 has changed in several physiological dysfunctions such as in stress induced depression (19), epilepsy (20), and cardiovascular dysfunctions (21). However, very little was present in the literature on the question of does serum HMGB1 levels changes depending on sex and age as a normal biological process. In a recent clinical study, it has been indicated that HMGB1 levels show differences depending on sex, age, and race, such as its levels are higher in females than in males, and in Blacks than in White. In addition, HMGB1 levels rise with increasing age (22). Similarly, the findings of the present study indicated that circulating levels of HMGB1 was affected by sex-by-age interaction, such as its level was higher in old females than in young females. In addition, it should be indicated that there was no difference in HMGB1 between young and old males, unlike females. On the contrary, there are conflicting evidence in the literature as to age- and sex- specific changes in the circulating levels of HMGB1. Data from a previous study stated that circulating level of HMGB1 exerted age-dependent change, indicated by the fact that serum HMGB1 levels was lower in healthy old human when compared to healthy young human (23). However, another clinical study has showed that HMGB1 levels do not show sex- and age-specific differences in healthy control (24).

HSPs are critical chaperon proteins induced by several factors, such as oxidative stress, inflammation, which play a crucial role in homeostasis, cell survival etc. Among HSPs, HSP72, HSP27, HSP90 and HSP60 are of great interest in extracellular compartment as alarmins. That means they take a part in the transmission of signal about alarm (25). Their cellular functions show differences; for example, HSP90 may exert anti-apoptotic features although HSP60 may be important in pro-apoptotic process (26). Their circulating levels may be assessed to be a potential marker for some diseases, including diabetes (27) and acute lymphoblastic leukemia (28). Therefore, it is important to identify age- and sex-specific alternation in circulating levels of HSPs for clarifying their role in health and disease. For example, as organism is getting older, decreased levels of HSPs may make maintaining homeostasis to difficult, resulting in biological dysfunctions-cancer and cell senescence etc. (29). In addition, several cellular process such as cell differentiation and cell cycle regulate HSPs synthesis under normal physiological function (30). In aging, circulating HSP70 became decrease in healthy population and with advance in age, it was found a positive association between HSP70, inflammation and frailty in older patients (30). A previous study indicated that circulating levels of HSP60 decreased as getting older (31), which supports available data emerging from the present study. In young males, circulating HSP60 levels were higher than in old males, and young females also had lower HSP60 levels than in young males. These results indicate that an effect of sex and sex-by-age interaction on circulating HSP60 levels exists in the current study, wherein circulating levels of HSP60 declined as

males got older. What is curious about this result is that age-related changes in circulating HSP60 was not observed in females. Akin to HSP60, a sex-by-age interaction had a significant effect on circulating HSP90, which indicated that circulating HSP90 appeared to decrease with age in males although it tended to increase with age in females. It should be emphasized that pair-wise comparison between groups did not reach a statistically significant level. Similarly, circulating HSP60 levels become increase after menopause in healthy women and no correlation between HSP60 and age as well as with how much years pass following menopause was observed. In addition, HSP60 levels were found to be increased in the situation of estrogen deprivation or deficiency following ovariectomy (32, 33). In peripheral blood cells, age-specific increase in the basal levels of HSP90 was obtained under normal physiological conditions (30). Circulating HSP90 levels showed a significant positive correlation with age in healthy subject while HSP90 showed a weak negative correlation with age in patients subjects regardless of sex (27). However, controversy data are present. In healthy young age-matched men and women, circulating levels of HSP60, HSP70 and oxidative stress markers such as reduced glutathione and thiobarbituric acid reactive substance did not indicate sex-dependent differences (34). Although tissue specific differences in the protein expression of HSP90 and HSP60 have been obtained (35), age- and sex-specific alteration in circulation HSP90 levels in healthy conditions has not yet been exactly clarified. As far as the author knows, it is the first study to explore this issue.

TLRs, found in almost all cells, are crucial proteins that recognize DAMPs. Based on their location in the cell, TLRs can be divided into two types, including cell membrane TLRs (TLR1, TLR2, TLR4, TLR5, and TLR10) and intercellular TLRs (TLR3, TLR7, TLR8, and TLR9). A surge in their expression results in excess activation of the inflammatory process (36). TLR2 and TLR4 have been suggested as a biomarker for many diseases, such as breast cancer (37), colorectal cancer (38), multiple sclerosis (39), acute aortic dissection (40), and diabetes (41). TLR2 and TLR4 are known as the soluble form of TLRs, which may act as an inhibitor for excessive TLR activation (36). In addition, signaling pathways initiated by TLRs activation might be negatively regulated by soluble form of TLRs found in the circulation. In other words, circulating TLRs or soluble TLRs act as a receptor for ligands which induces activation of TLRs, thereby limiting binding of the ligands to TLRs (42). This means that the inflammatory process mediated by TLRs might be prevented by soluble TLRs in the circulation. In the skin sample, the expression profile of TLRs has shown differences depending on age, indicated that TLR2 and TLR4 expression in adult skin specimen was lower than in embryonic and fetal one (43). Salivary concentration of TLR2 decreased with age, as evidenced by healthy individuals aged 30-39 years having lower TLR2 levels than those aged 6-15 years (44). It should be emphasized that it was not focused on sex-related changes in salivary concentration of TLR2 in the mentioned study. Overall, these results state importance of age and sex in the levels of TLR2 in different body fluid. In support of these results, the result of the current study indicated that a sex- and age-specific differences in TLR2 and a sex- and age-by-sex interaction in TLR4 levels was observed, wherein circulating TLR2 level was lower in old females than in young females, and TLR2 and TLR4 in young females was high compared to young males. Interestingly, age-related decrease in TLR2 and TLR4 were not markedly observed in males. Females have higher TLRs expression on macrophage, estrogen deficiency following ovariectomy results in decreased expression of TLR2 and TLR4 on macrophages (45). In another study, it has been showed that expression of TLRs on platelet was higher in women than in men (46). In contrast, conflicting evidence is also present on sex-specific differences in circulating TLR4 level, indicated by a clinical study reported that serum TLR4 levels did not exert difference between males and females from control groups (40).However, it has been unknown whether estrogen levels can modulate cleavage and/or ectodomain shedding of surface TLRs, resulting in increased serum TLRs levels. Although serum estrogen levels were not measured in the present study, it may be speculated that estrogen may be responsible for higher circulating levels of TLRs because 24 months old female mice had lower serum TLR2 than 6 months old female mice. Further study is required to assess the role of estrogen level in circulating TLRs and their association in health and disease state. In addition, age-specific changes in the function of TLRs are not exactly clarified (47); however, changes in circulating levels of TLRs may be given as a possible explanation for such alteration in their function.

The cellular mechanism underpinning HMGB1 release and/or activity have not yet exactly clarified. Oxidative stress may be an important factor for the functions of HMGB1 (48, 49). Treatment of experimental animals with lipopolysaccharide resulted in increased reactive oxygen generation, which in turn induced the release of HMGB1 into circulation. That means a link between oxidative stress and HMGB1 release (50). In old females, there was a likely positive correlation between TOS and HMGB1. However, the observed difference between TOS and HMGB1 in this study was not significant (r = 0.740, p = 0.057). HMGB1 is also crucial in depressive behavior. Serum levels of HMGB1 increased in stress induced depression. It has been proposed that

the underlying mechanism of HMGB1 in depressive behavior might be mediated via TLR4, which led to cytokine induction (19). In epilepsy patients, there was a positive correlation of HMGB1, TLR4 with epilepsy seizure as well as higher serum HMGB1 and TLR4 levels than healthy subjects (20). In addition, a current study has been set out to determine whether circulating levels of HGMB1 is an important factor in epilepsy patients' resistance to drugs (51). With respect to the question, it is found that serum levels of HGMB1 increase in patients with drug resistance although there is no association between HMGB1 and seizures, by which it is suggested that targeting circulating HMGB1 may be a good point for evaluating the therapy efficacy in the epilepsy treatment. It should be mentioned that age- and sex-specific differences in this correlation were not evaluated in these clinical studies. In reviewing the literature, no data was found on the association between HMGB1 and age and sex under normal physiological conditions. In the present study, a significant positive correlation between HMGB1 and TLR2, TLR4 and HSP60 only in young females was observed. In accordance with a previous study, there was a significant correlation between TLR2 and HMGB1 levels and a trend towards a positive correlation between TLR4 and HMGB1 with no reaching significant levels (52). HSPs can circulate throughout the body via bloodstream, and they exert cellular effects via cell surface receptors. HSPs are known as endogenous ligands for TLRs, especially TLR2 and TLR4 (53). For example, HSP60 induced NF-κB signaling pathway by binding to TLR2 or TLR4, resulting in production of cytokines and chemokines (54, 55). In addition, HSP60 induced expression of TLR2 (33). In old males, a negative correlation was found between HSP60 and TLR2 in the present study. In addition, a negative correlation was found between HSP90 and TLR4 in young males. In contrast, it was found that HSP60 was positively correlated with TLR4 and a correlation between TLR2 and HSP60 levels with no reaching significant levels (52). The negative correlation between HSP60 and OSI in old males is interesting because general knowledge is that oxidative stress result in expression of HSPs, especially HSP90 and HSP60. The reason for such controversy may arise from that in response to oxidative stress, circulating levels of HSPs may differently change when compared to their protein expression in a cell or tissue.

In the current study, the mechanism by which age and sex leads to changes in the serum levels of mentioned parameters was not addressed here but remains an interesting avenue for further study. Both transcriptional and post-transcriptional mechanisms should be investigated. Circulating levels of HMGB1, HSPs and TLRs with aging and sex differences. It should be kept in mind that the presence or absence of correlation does not explain causality. Therefore, further works should be designed to elucidate the causal mechanisms underlying associations between these parameters based on sex and age. The findings of this study provide important insights into the role of age and sex in circulating levels of parameters studied and make contributions to the current literature. Therefore, the present study lays the groundwork for future research on these subjects.

CONCLUSION

Scientists are increasingly researching age-related cellular changes and the physiological dysfunctions caused by these changes to produce new treatment approaches and solutions. The results indicated that age-related increases in oxidative stress varied between sexes and tended to be greater in females, as demonstrated by higher levels of TOS and OSI in old females. TLR2, TLR4 and HSP60 indicated differences between sexes and females had higher levels of TLR2 and TLR4, and lower level of HSP60 in circulation. Moreover, HMGB1 increased in the circulation as females, but not males, got older. These data might provide the basis information on the role of sex and age in variation of circulating levels of HSPs, TLRs and HMGB1. An understanding of age- and sex-related differences in HMGB1, HSPs and TLRs as well as their interaction under normal biological process as biomarkers is critical to develop new therapies to ameliorate disease and/or to clarify differentiation in the cellular pathways associated with aging and biological sex.

Ethics Committee Approval: All experiments were performed with the permission of the Local Animal Ethics Committee of Düzce University, Düzce, Türkiye (Approval no: 2021/03/06).

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RESEARCH ARTICLE

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Investigation of the Relationship Between Vitamin D and Peripheral Inflammatory Parameters in Children with Attention Deficit and Hyperactivity Disorder

ABSTRACT

Objective: We aimed to investigate the effects of vitamin D and some nutritional factors such as vitamin B12, folate, homocysteine, and ferritin, which play a role in the pathogenesis of attention deficit and hyperactivity disorder (ADHD), on inflammation, which is also claimed to play a role in the pathogenesis of ADHD.

Method: 39 ADHD and 39 healthy controls were compared with similar age, gender and BMI. The severity of the disease was evaluated with the Turgay ADHD scale. Inflammatory and nutritional parameters were measured routinely.

Results: In the patient group, Vitamin D was found to be significantly lower ($p<0.001$), while homocysteine was found to be significantly higher ($p=0.003$). CRP and MPV values among inflammatory parameters were found to be significantly higher ($p<0.001$ for both). No significant correlations were found between nutritional factors and inflammatory parameters ($p>0.05$ for all).

Conclusion: It can be suggested that low levels of vitamin D and high levels of homocysteine, which is related to one carbon metabolism, may play a role in the pathogenesis of ADHD. High levels of some inflammatory values may also indicate the role of inflammation in the pathogenesis of ADHD. No significant relationship was found between nutritional and inflammatory parameters. However, considering the limitations of the study, further research is needed on this subject.

Keywords: Vitamin D, Nutrition, Inflammation, Peripheral Inflammatory Markers, Attention Deficit and Hyperactivity Disorder, One-Carbon Metabolism.

Dikkat Eksikliği ve Hiperaktivite Bozukluğu Bulunan Çocuklarda Vitamin D ile Periferal İnflamatuar Parametreler Arasındaki İlişkinin Araştırılması

ÖZET

Amaç: Dikkat eksikliği ve hiperaktivite bozukluğu (DEHB) patogenezinde rol oynayan vitamin D ile vitamin B12, folat ve homosistein, ve ferritin gibi bazı nütrisyonel faktörlerin, yine DEHB patogenezinde rol oynadığı ileri sürülen inflamasyon üzerine olan etkileri araştırılmasını hedefledik.

Gereç ve Yöntem: 39 DEHB bulunan ve 39 sağlıklı kontrol grubu yaş, cinsiyet ve BMI benzer olacak şekilde karşılaştırıldı. Turgay DEHB ölçeği ile hastalığın şiddeti değerlendirildi. Rutin olarak inflamatuar ve nütrisyonel parametreler ölçüldü.

Bulgular: Hasta grubunda, Vitamin D anlamlı olarak düşük bulunurken ($p<0.001$), homosistein ise anlamlı olarak yüksek bulunmuştur ($p=0.003$). İnflamatuar parametreler içinde CRP ve MPV değerleri anlamlı olarak yüksek bulunmuştur (ikisi için $p<0.001$). Nütrisyonel faktörler ile inflamatuar parametreler arasında anlamlı korelasyonlar tespit edilmemiştir (hepsi için $p>0.05$).

Sonuç: Vitamin D'nin ve tek karbon metabolizması ile ilgili olan homosistein yüksekliğinin DEHB patogenezinde rol oynayabileceği düşünülebilir. Bazı inflamatuar değerlerin yüksek olarak bulunması da DEHB patogenezinde inflamasyonun rolüne işaret edebiliri. Nütrisyonel ve inflamatuar parametreler arasında anlamlı bir ilişki bulunmamıştır. Fakat çalışmanın kısıtlılıkları göz önüne alındığında bu konuda ileri araştırmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Vitamin D, Nütrisyon, İnflamasyon, Periferal İnflamatuar Belirteçler, Dikkat Eksikliği ve Hiperaktivite Bozukluğu, Tek-Karbon Metabolizması.

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INTRODUCTION

Attention deficit and hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of childhood period. ADHD is a disorder characterized by clinical symptoms such as inattention, impulsivity, and hyperactivity that begin before the age of 12 (1). Its incidence is around 5.29% worldwide (2). Despite numerous studies, the pathogenesis of ADHD has not been fully elucidated. Although no specific cause has been identified, ADHD is a multifactorial disorder (3).

Regarding single carbon metabolism, vitamin B12, folate and homocysteine may contribute to many childhood and adolescence psychiatric disorders. It has been reported that these factors may have an effect on neuropsychiatric diseases such as depressive disorder, obsessive-compulsive disorder, ADHD, special learning disability and autism spectrum disorder (4-7). One-carbon metabolism (OCM), including these vitamins, plays a role in DNA synthesis as well as in the synthesis of neurotransmitters such as serotonin and dopamine. Besides, the synthesis of S-adenosyl methionine (SAM) which is the universal methyl donor, is formed by these metabolic reaction chains. SAM provides normal functioning of epigenetic mechanisms by taking part in DNA methylation together with methylation of many proteins. Therefore, gene expressions are negatively affected in situations of vitamin B12 and folate deficiency in which SAM production decreases (8). In addition, homocysteine, which increases as a result of vitamin B12 and folate deficiency, is an extremely neurotoxic substance. Besides, it damages the vascular endothelium. Increase in homocysteine is one of the most important parameters indicating vitamin B12 and folate deficiency (9). It has also been reported that the polymorphism of the enzyme methyltetrafolate reductase, which is one of the OCM enzymes, plays a role in the etiology of ADHD (10).

After it was understood that vitamin D has many effects other than bone metabolism, there has been an explosion in the studies that vitamin D may play a role in psychiatric diseases in recent years. There are many publications showing that it has an effect on pathogenesis of neuropsychiatric disorders in childhood and adolescence period(4-7). These effects of vitamin D have been particularly interesting as it has been shown that they are involved in the synthesis of neurotransmitters such as serotonin and dopamine (11,12). Vitamin D takes part in mechanisms necessary for the development of the brain and the continuation of its normal functions, such as neurogenesis, neuroplasticity, myelination, and neuroprotection (13). In addition to all these neurological effects, vitamin D has anti-inflammatory, autoimmunity suppression and protective effects against oxidative stress (14).

In addition to being a good indicator of iron stores, ferritin is known as an acute phase reactant that increases in the inflammatory response (Koorts 2011). It has been shown that low ferritin levels are associated with ADHD symptoms (Oner 2008). Ferritin has a protective effect against microbial proliferation, oxidative damage and inflammation by removing intracellular iron and storing it during inflammation (15).

Irregularities in the immune system and inflammation have been found to be effective in the pathogenesis of ADHD (16). Hemogram values, CRP and sedimentation evaluations, which are simple inflammatory parameters (SIP), are routine measurements that are easily performed and cost-effective. Neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio and platelet-to-lymphocyte ratios are obtained by dividing the hemogram values. Besides being easy and inexpensive, they are good markers of inflammation (17).

There are evidences that there may be a relationship between inflammation and nutritional parameters such as vitamin B12, folate, vitamin D, homocysteine and ferritin, which are effective in the evaluation of nutritional status (18). It has been shown in an animal study that vitamin B12 may have an anti-inflammatory effect (19). In addition, it has been found that hyperhomocystinemia, which occurs as a result of vitamin B12 and folate deficiency, is associated with inflammatory processes (20,21). Again, in a recent animal study, it was shown that hyperhomocysteinemia cause increased pro-inflammatory cytokines and decreased anti-inflammatory cytokines in brain and retinal cells (22). In addition to these effects of vitamin B12 and folate on inflammation, it has been found that many immune cells carry Vitamin D receptors. Therefore, these findings indicate that vitamin D has effects on the immunological response (23). Vitamin D shows anti-inflammatory properties. Vitamin D reduces the expression of proinflammatory cytokines such as TNF-alpha, IL-1β, IL-6 and IL-8 (24).

In this study, considering the relationship of nutritional parameters such as vitamin B12, folate, homocysteine, vitamin D and ferritin with the inflammation, the hypothesis that there may be an interaction between these vitamins and inflammation in ADHD pathogenesis was investigated.

MATERIAL AND METHODS

Ordu University Medical Faculty Hospital Child and Adolescent Psychiatry Clinic conducted this study. The patients who applied to our clinic and diagnosed ADHD formed patient group. ADHD diagnosis were made according to DSM 5 criteria. A healthy control group was formed from the subjects who came to the healthy child follow-up outpatient clinic in the pediatrics department

without any medical or psychiatric diagnosis. Psychiatric diagnoses were generally investigated by applying K-SADS-PL to all subjects. Subjects were included in the study as a result of detailed psychiatric examination, family interviews and medical record reviews by the child psychiatrist. Subjects with ADHD were administered the Atilla Turgay ADHD scale to assess the severity of the disorder. In addition to these, sociodemographic data form was filled in all subjects. Nutritional and inflammatory parameters were measured routinely in the hospital laboratory. Blood samples were taken between 08:00 and 11:00 in the morning before breakfast. Under steril conditions, samples were taken from antecubital vein. Exclusion criteria were those with a history of systemic diseases, obesity, acute and chronic infections, nutritional support in the last one year, and those with epilepsy and neurological deficits. Ethics Committee of Ordu University Faculty of Medicine approved the study (Decision no: 2021/225).

Sociodemographic Form: With this form, information such as age, height, weight, BMI, medical history, family history, birth history, breastfeeding time, and drug use information were obtained.

Schedule for Affective Disorders and Schizophrenia for School-Age Children– Present and Lifetime Version DSM 5 (K-SADS-PL DSM 5): This scale is arranged according to DSM 5. Determines whether diagnoses found according to DSM 5 in children and adolescents are present now and throughout life. In this scale, psychiatric symptoms are determined. There is also a further list of symptoms. Here, a psychopathological evaluation is made. This assessment scale has Turkish validity and reliability (25).

Turgay DSM-IV-Based Child and Adolescent Behavior Disorders Screening and Rating Scale: Turgay developed this scale according to DSM-IV criteria. 9 items determines attention deficit. 6 items questions the symptoms of hyperactivity. While 3 items evaluate impulsivity, 8 items determine oppositional defiant disorder. Conduct disorder is evaluated in 15 items. In each item, the degree of symptom is determined as 0-1-2-3. This scale has validity and reliability in Turkish (27).

Blood Tests: Hemogram parameters were determined with the Abbott CELL-DYN 2700 device (Abbott Laboratories, Illinois, USA). CRP levels were measured with the immunoturbidimetry method (Archem Diagnostic Industry, Istanbul, Turkey). The Architect i1000 analyzer measured 25-OH-Vitamin D levels (Abbott Laboratories, Abbot Park, Illinois, USA). This measurement was made using the chemiluminescent microparticle immune study method. Homocysteine levels were measured in accordance with Abbott Laboratory commercial kits. The chemiluminescent immunoassay method was used. Again, vitamin b12 was measured with the same commercial company kits. Chemiluminescent micro particle Intrinsic Factor ARCHI-TECT B12 method was used. In addition, folate was determined with the same method and kits.

Statistics: SPSS 22.0 software program was used for data analysis. Whether the numerical variables were normally distributed or not was evaluated with the Shapiro-Wilk test. Normally distributed numerical variables are shown as mean±SD, while non-normally distributed variables are shown as median (IQR). Chi-square test was used to compare categorical variables. Student-t test was used to compare normally distributed variables. Mann Whitney-U test was used to compare variables that show non-normally distribution. We accepted P values below 0.05 as significant.

RESULTS

The groups are almost completely similar to each other in terms of gender and age. BMI comparisons between two groups showed no significant differences (Table 1). Vitamin B12 and folate were found to be insignificantly similar between the two groups. On the other hand, while vitamin D was found to be low in the patient group, homocysteine was found to be high in the patient group. These differences found to be significant. Ferritin values also did not differ between the two groups (Table 1).

Table 1. Characteristics of groups and values of nutritional factors

	Patients Group (n=39)	Healthy control group (n=39)	P value
Gender (Female/Male)	6/33	6/33	1[a]
Turgay ADHD scale (median) (IQR)	54 (18)	-	-
Age (median) (IQR)	8 (3)	7 (4)	0.916[b]
BMI (kg/m2) (median) (IQR)	17.08 (1.77)	16.82 (2.47)	0.168[b]
Vitamin B12 (pg/ml)(mean±SD)	402.74±141.67	438.07±190.72	0.356[c]
Folate (ng/ml) (mean±SD)	8.87±3.43	8.23±2.87	0.378[c]
Vitamin D(ng/ml) (median) (IQR)	20.8 (8.1)	28.5 (9.6)	<0.001[b]
Homocysteine (umol/L) (median) (IQR)	10.10 (4)	8.60 (1)	0.003[b]
Ferritin (ug/L)(mean±SD)	35.67±12.10	28.91±19.48	0.189[c]

Footnot: a.Chi-square test b.Mann Whitney-U test c.Student t test

Hemogram parameters such as neutrophil, lymphocyte, monocytes and platelet counts were similar between the two groups. MPV values were found to be significantly higher in the patient group. Although NLR values were found to be high in the patient group, this difference was found to be statistically insignificant. However, this value was found close to the significance limit (p=0.092). MLR and PLR values did not differ. Likewise, sedimentation values did not differ. CRP levels were determined higher in the patients group and this was statistically significant (p<0.001) (Table 2). Demographic data is 2 (age, BMI), nutritional values are 5 (vitamin B12, folate, vitamin D, homocysteine and ferritin), and inflammatory and hemogram parameters are 10 (neutrophil, lymphocyte, monocytes, platelet, MPV, NLR, MLR, PLR, CRP and sedimentation), their correlation analysis results have been obtained in large numbers. Since presenting all of them would take up a lot of space for an article, a general evaluation was made. As a result, when these reciprocal correlation analyzes were evaluated, no significant result was obtained between demographic and nutritional factors, inflammatory and hemogram parameters (p>0.05 for all). Only as expected, significant correlations were found between homocysteine and vitamin B12 (r=-0.386 and p=0.01) and between ferritin and sedimentation (r=0.458 and p=0.003). No correlation was found between T-DSM-IV-S, which shows ADHD severity, and both nutritional and inflammatory parameters (p>0.05 for all).

Table 2. Distribution of simple inflammatory parameters by groups

	Patients Group (n=39)	Healthy control group (n=39)	P value
Neutrophil	3010 (1480)	2800 (1370)	0.303[a]
Lymphocyte	2740 (940)	2730 (1350)	0.566[a]
Monocyte	470 (160)	490 (220)	0.853[a]
Platelet	320.28±72.50	316.97±72.15	0.841[b]
MPV	9.4 (1)	7.42 (1.25)	<0.001[a]
NLR	1.08 (0.4)	0.907 (0.372)	0.092[a]
MLR	0.181±0.551	0.173±0.057	0.529[b]
PLR	113.29 (42.49)	108.02 (52.49)	0.382[a]
CRP (mg/dL)	0.06 (0.14)	0.02 (0.04)	<0.001[a]
Sedimentation (1 hour)	8 (6)	8 (11)	0.093[a]

Footnote: MPV: mean platelet volüme; NLR:Neutrophil-Lymphocyte Ratio; MLR:Monocyte- Lymphocyte Ratio; PLR:Platelet-Lymphocyte Ratio; CRP:C-Reactive protein
a.Mann-Whitney U test b.Student t test

DISCUSSION

This study is the first study in the literature investigating the effects of nutritional factors such as vitamin D, vitamin B12, folate, ferritin and homocysteine on simple inflammatory parameters in ADHD patients. Since ADHD is the most common psychiatric disorder in childhood and has many negative consequences, understanding its etiopathogenesis may contribute to the discovery of new treatment options. According to the results obtained, vitamin D among nutritional factors was found to be significantly lower in the patient group. However, homocysteine levels, which are one of the parameters that best show vitamin B12 and folate deficiency, were found to be high in the patient group. When evaluated in terms of inflammatory parameters, NLR was found to be high in the patient group, which was close to statistical significance. In addition, CRP values were found to be high in the patient group. However, no significant effect of all these nutritional factors on SIPs was detected.

It has been reported that decreased vitamin B12 and folate and related increase in homocysteine are effective in many childhood psychiatric disorders (6,28). Indeed, these vitamins are essential for maintaining healthy brain functions. The OCM, of which they are a part, plays a fundamental role in DNA synthesis, neurotransmitter maintenance, and methylation reactions. Although these vitamins were not found to be low in this study, homocysteine was found to be increased. In fact, the increase in homocysteine is one of the best parameters for these vitamin deficiencies. In the results of this study, an inverse correlation was found between vitamin B12 and homocysteine. However, while these vitamins are normally measured in the blood, they can be found in lower levels in the brain tissue (29). Accordingly, brain tissue may have relatively lower vitamin levels. Increased homocysteine may contribute to the pathogenesis of ADHD. Normal vitamin B12 and folate levels were found to be compatible with some previous studies (30).

Vitamin D is necessary for healthy brain functions and is considered as a risk factor in the etiology of many psychiatric disorders. In the last meta-analysis study, it was shown that vitamin D levels were found to be low in children with ADHD (31). In our study, vitamin D levels were found to be low, in line with these results. Vitamin D has

also been reported to play a role in healthy immune system functioning. It has been shown recently that vitamin D deficiency may also play a role in ASD patients and that vitamin D is associated with inflammatory parameters (32). However, no relationship was found between vitamin D and SIP in this study.

There are studies showing that ferritin is associated with ADHD symptoms (33). Ferritin and thus iron metabolism are essential for monoaminergic neurotransmission (34). There are studies reporting that ferritin levels do not differ in adhd patients (30). These reports are consistent with our results.

There are many studies pointing to the association of inflammation and ADHD (35). SIP are parameters that show inflammation, are easily and inexpensively obtained and easily calculated. These parameters have been studied in ADHD patients so far, and some of them have been found to have high indicators such as NLR (36). No significant elevations of SIP were found in this study. However, NLR was found to be high in the ADHD group, close to significance. However, MPV, which is also an indicator of inflammation, and CRP, which is considered an acute phase reactant, were found to be high in the ADHD group. These results are consistent with studies indicating that inflammation plays a role in the pathogenesis of ADHD. However, no effect of nutritional values on these parameters was detected.

Some limitations in this study restrict making a more accurate interpretation. The number of subjects is too small. So, this constitutes serious limitation. The model of the study is cross-sectional. This study models are insufficient to explain the cause-effect relationship. In order to make a better assessment of the relationship between inflammation and nutritional factors, inflammatory mediators such as TNF alpha, IL-6, and IL-1 must be measured and evaluated. And this provides us to make a more accurate explanation.

CONCLUSION

It can be suggested that low vitamin D and high homocysteine levels may contribute to the pathogenesis of ADHD. However, high inflammation parameters such as CRP and MPV support the association of inflammation-ADHD. However, no relationship was found between nutritional values and inflammation. It seems clear that further research is needed on this subject.

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RESEARCH ARTICLE

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Do We Need to Repeat the Initially Normal Head Computerized Tomography for Patients with Mild Head Trauma Using Anticoagulant and/or Antiplatelet Therapy?

ABSTRACT

Objective: Patients using anticoagulant and/or antiplatelet (AC/AP) medications are at an increased risk of intracranial hemorrhage (ICH) subsequent to head trauma and current guidelines recommend a head computed tomography (CT) scan for these patients. There is a lack of consensus about management recommendations for mild head trauma patients on AC/AP treatment who had an initially normal head CT. The aim of this study was to determine the rate of delayed ICH after a 24-hour observation in patients with mild head trauma using AC/AP who had an initially normal head CT.

Method: Patients aged 18 and older, using AC/AP drugs with mild head trauma were included prospectively. Patients underwent head CT for suspected bleeding. A repeat CT scan was performed after a 24-hours observation period for the patients who had an initially normal head CT for detecting delayed intracranial hemorrhage.

Results: A total of 101 patients were included and, 57.4% (n=58) of the patients were female. Delayed ICH was detected in 2.9% (n=3) of the patients after a 24-hour observation. None of the patients with delayed ICH needed surgical treatment or further intervention. Delayed ICH was found in patients who used acetylsalicylic acid (n=1), dabigatran (n=1), and apixaban (n=1).

Conclusion: In patients with mild head trauma using AC/AP, delayed intracranial hemorrhage is rare and may be clinically insignificant. A repeat CT scanning after 24-hour observation may not be necessary for patients with mild head trauma using AC/AP therapy who had initially normal head CT.

Keywords: Anticoagulant Drugs, Antiplatelet Drugs, Head Trauma, Intracranial Hemorrhage.

Antikoagülan ve/veya Antiplatelet Tedavi Kullanan Hafif Kafa Travmalı Hastalarda Başlangıçta Normal olan Bilgisayarlı Kafa Tomografisini Tekrarlamamız Gerekir mi?

ÖZET

Amaç: Antikoagülan ve/veya antiplatelet (AC/AP) ilaç kullanan hastalarda kafa travması sonrası intrakraniyal kanama (İKK) riski yüksektir ve güncel kılavuzlar bu hastalar için bilgisayarlı kafa tomografisi (BT) görüntülemesini önermektedir. Başlangıçta normal kafa BT'si olan ve AC/AP ilaçları kullanan hafif kafa travmalı hastalar için yönetim önerileri konusunda fikir birliği yoktur. Bu çalışmanın amacı, başlangıçta normal kafa BT'si olan ve AC/AP kullanan hafif kafa travmalı hastalarda 24 saatlik bir gözlem sonrasında gecikmiş İKK oranını belirlemektir.

Gereç ve Yöntem: AC/AP ilaç kullanan, 18 yaş ve üzeri hafif kafa travmalı hastalar prospektif olarak çalışmaya alındı. Hastalara kanama şüphesi nedeniyle kafa BT'si çekildi. Çekilen ilk kafa BT'si normal olan hastalara gecikmiş intrakraniyal kanamayı saptamak için 24 saatlik gözlem süresi sonrasında tekrar kafa BT görüntülemesi yapıldı.

Bulgular: Toplam 101 hasta çalışmaya dahil edildi ve hastaların %57,4'ü (n=58) kadındı. 24 saatlik gözlem sonrasında hastaların %2,9'unda (n=3) gecikmiş İKK saptandı. Gecikmiş İKK'li hastaların hiçbirinde cerrahi tedavi veya başka girişim gerekmedi. Gecikmiş İKK, asetilsalisilik asit (n=1), dabigatran (n=1) ve apiksaban (n=1) kullanan hastalarda saptandı.

Sonuç: AC/AP kullanan hafif kafa travmalı hastalarda gecikmiş İKK nadirdir ve klinik olarak önemli olmayabilir. Başlangıçta kafa BT'si normal olan, AC/AP ilaç kullanan hafif kafa travması olan hastalarda 24 saatlik gözlemden sonra kafa BT görüntülemesinin tekrarı gerekli olmayabilir.

Anahtar Kelimeler: Antikoagülan İlaçlar, Antiplatelet İlaçlar, Kafa Travması, İntrakraniyal Kanama.

INTRODUCTION

Head trauma is one of the common reasons for emergency department (ED) admissions. In the United States of America, over 2.5 million head trauma patients present to EDs annually (1). Over the years, the number of patients admitted to the ED with head trauma has increased in all age groups, though the largest increase is in patients over 60 years old (2). Anticoagulant and antiplatelet (AC/AP) drugs are of critical importance in the treatment protocols of diseases such as coronary artery disease and cardiac arrhythmia and the incidence of use of these drugs increases with age (3).

One of the important risk factors for intracranial hemorrhage (ICH) in patients with head trauma is the use of AC/AP. Delayed ICH is defined as the finding of a normal brain computerized tomography (CT) after head trauma with a repeat CT demonstrating ICH without subsequent trauma. Delayed ICH may be seen in 0.6% to 6.2% of patients with head trauma (4-11).

Current guidelines recommend an initial CT in patients on AC/AP presenting with head trauma (12-14). However, there is no consensus on recommendations for the follow-up of patients with mild traumatic brain injury (TBI) in terms of the observation period and routine repeat CT for delayed ICH (6-8). Among studies, three recommendations have emerged for the management of patients with mild TBI; The first one is to observe these patients for 6 to 24 hours after the first CT presenting no ICH (4,6,9), the second one is to perform a repeated CT after the time of observation (7,11,15), and the third one suggests discharging patients without clinical observation or a repeat CT (10,16). The management of these patients group remaining unclear and causes confusion among physicians. The aim of this study was to determine the rate of delayed ICH after a 24-hour observation in patients with a mild head trauma using AC/AP who had an initially normal head CT.

MATERIAL AND METHODS

Study Design: This was a prospective observational cross-sectional descriptive study. Initially, a head CT scanning without contrast-enhanced was performed in patients with head trauma according to NEXUS Head CT Instrument. CT scans were interpreted by the neuroradiologist in charge at the time. Patients with a negative initial head CT were observed in the ED for 24 hours from the time of hospital admission. If neurological deterioration (decrease in GCS, new neurological deficits, seizure) developed during the observation period, a repeat CT scan and a neurosurgical consultation was planned. For patients without neurological deterioration or worsening symptoms during the 24-hour observation period, a repeat head CT was performed at the end of the observation period. Patients with delayed ICH on repeat head CT were consulted to the neurosurgery department. Patients with no further complications were discharged from ED with instructions. Follow-up was extended up to one month and patients were called on one week and 30 days later after trauma. One month after the head trauma, the patients were invited to ED for evaluation of trauma impact, and a detailed neurological examination was performed but the brain CT was not repeated. It was questioned whether there were any neurological symptoms related to head trauma and whether patients were re-admitted to the hospital one-month period after head trauma.

Setting: The study was conducted in the ED of a university hospital with an annual ED presentation of 55,000 patients. Patients with blunt head trauma using any AC/AP medication presented to the ED between February 1, 2019, and January 31, 2020, were included in the study. Ethical approval was received from the Clinical Research Ethics Committee of the Ankara University School of Medicine with the approval number 02-114-19 and date of 28.01.2019.

Participants: All patients with blunt head trauma using any AC/AP medication presented to the ED between study dates were assessed for eligibility. The inclusion criteria were; patients aged ≥18 years, had a Glasgow Coma Scale (GCS) score of 13-15, had taken AC/AP drugs in the last 24 hours, and was admitted to the ED within the first 2 hours after trauma (14). We excluded patients referred from another health institution, and pregnant patients. Patients with ICH or skull fracture on initial CT were excluded from the study. In case the patients had other trauma in addition to the head trauma, appropriate diagnostic imaging modalities were performed for the affected body structure. Patients who did not accept the observation in the ED or declined a repeat head CT after the observation were excluded from the study. Written informed consent was obtained from all patients included in the study.

Variables: Patients who met the study inclusion criteria were enrolled in the study and patient information was recorded on the study form prepared in advance. Demographic characteristics, GCS score, mechanism of trauma, type of AC/AP drugs were collected for each patient. Complete blood count, activated partial thrombin time (aPTT), and international normalized ratio (INR) tests were performed to evaluate the bleeding risk of the patients. During the observation period, patients were monitored, and re-evaluated every two hours, vital signs (fever, pulse, respiratory rate, blood pressure) and GCS scores were recorded.

The primary outcome was to assess the presence of delayed ICH in mild TBI patients using AC/AP on the repeat CT scan. The secondary outcomes were 30 day-mortality after head trauma

and determining risk factors associated with delayed ICH.

Sample Size: The prevalence of ICH was accepted %4 and estimated with a marginal error of %3.5, and with a type 1 error of %5, at least 119 patients with at least 5 ICH needed to be included in to study.

Statistical Methods: For descriptive statistical evaluations, mean ±standard deviation was used for continuous variables, frequencies and percentages were used for categorical variables. For comparison of variables between two independent groups; the t-test, the significance test of the difference between two percentages, and the Mann-Whitney U test was used. Chi-square Test and Fisher's Exact Test were used to examine the relationship between categorical variables. A p value <0.05 was considered significant. SPSS 23.0 package program was used for statistical analysis.

RESULTS

A total of 1276 patients with mild head trauma were admitted to the emergency department during the study period, 171 of these patients were using AC/AP drugs, and 1105 of these patients were not using any AC/AP drugs. Patients with mild head trauma using AC/AP drugs were evaluated for eligibility (n=171) and 70 of patients were excluded. A total of 101 patients who had a normal initial head CT, were observed 24 hours in the emergency department and had a repeat head CT after observation included for the analysis (Figure 1).

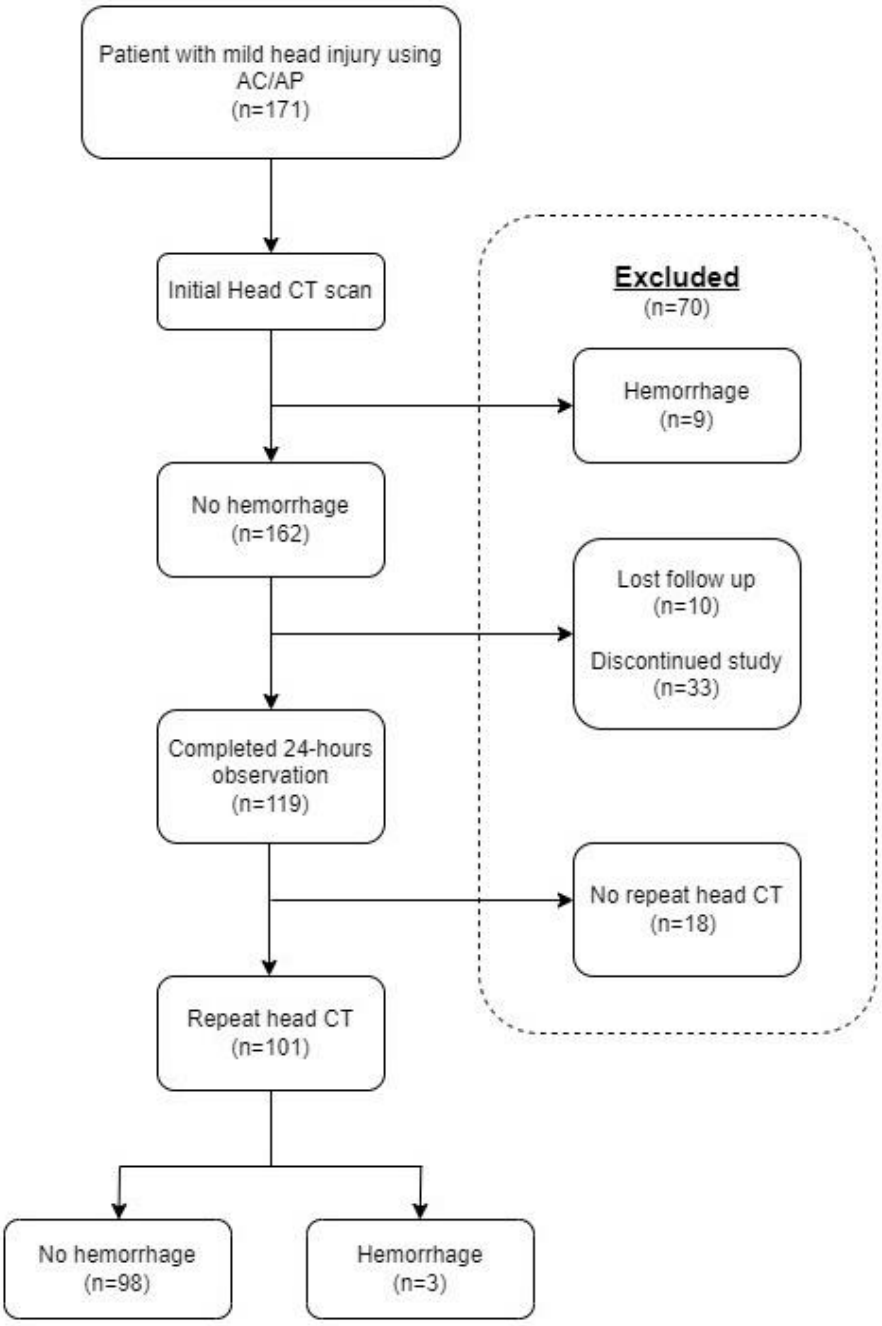

Figure 1. Flowchart of the patients and study protocol

The mean age was 75.52 ±10.01 years, and 57.4% (n=58) of these patients were women. The GCS score of 15 was found 99% of the patients (n=100). The most common trauma mechanism was ground-level fall (Table 1). Acetylsalicylic acid (ASA) (34.7%, n=35), clopidogrel (18.8%, n=19) and warfarin (9.9%, n=10) were found to be the most common AC/AP drugs. During the follow-up in the ED, no pathological changes in neurological examinations and no new symptoms were recorded in all patients. At the end of the 24-hour observation period, delayed ICH on repeat head CT was detected in 3 (2.9%, 95%CI: 0-6.3) of the patients. Factors reviewed for correlation to delayed ICH are shown in Table 2. The mean age of the three patients with delayed ICH was 78.66 ±8.5 years. None of them had new symptoms or neurologic deterioration in 24-hour observation. The clinical features of the patients with delayed ICH are shown in Table 3. Patients with delayed ICH were consulted to the neurosurgery clinic, none of them need further hospitalization, surgical intervention, or specific treatment, and they were all discharged with instructions. The follow-ups by telephone reported no complications.

Patients without delayed ICH on repeat CT (n=98) were discharged with instructions. Of the 94 patients who were reached by phone call did not have any complaints due to head trauma, and no mortality was reported at the end of the one-month period. Four of the patients could not be reached by phone call and the national health system was examined for these patients. It was observed that none of the patients was readmitted to the hospital within a one-month period and no mortality was noted.

Table 1. Demographic characteristics of the study population

Characteristics	**Number (%)**
Age, mean (±SD)	75.52±10.01
Gender	
Female	58 (57.4)
Mechanism of injury	
Ground-level fall	94 (93.1)
Traffic accident	6 (5.9)
Direct impact	1 (1)
GCS (on arrival)	
14	1 (1)
15	100 (99)
AC/AP	
Acetylsalicylic acid	35 (34.7)
Clopidogrel	19 (18.8)
Ticagrelor	5 (4.9)
Prasugrel	3 (3)
Warfarin	10 (9.9)
LMWH	8 (7.9)
Dabigatran	8 (7.9)
Rivaroxaban	9 (8.9)
Apixaban	9 (8.9)
Edoxaban	6 (5.9)
Combined use of AC/AP	11 (10.9)
Reason for AC/AP treatment	
Coronary artery disease	40 (39.6)
Atrial fibrillation	31 (30.6)
Stroke	13 (12.8)
Thromboembolic disease	8 (7.9)
Peripheral artery disease	6 (5.9)
Other	3 (2.9)
Total population	101(100)

GCS, Glasgow Coma Scale; LMWH, Low Molecular Weight Heparin; AC/AP, Anticoagulant and antiplatelet

Table 2. Factors affecting delayed ICH in repeat CT imaging

	No Delayed ICH (n=98)	**Delayed ICH (n=3)**
Age (years)	75.42 ± 10.08	78.66 ± 8.5
Gender*		
Female	58 (100%)	0 (0%)
Male	40 (93%)	3 (7%)
GCS score		
14	1 (100%)	0 (0%)
15	97 (97%)	3 (3%)
Mechanism of injury		
Ground-level fall	91 (96.8%)	3 (3.2%)
Traffic accident	6 (100%)	0 (0%)
Direct impact	1 (100%)	0 (0%)
Platelet count (10^9/L)*	231.5 ± 81.9	163 ± 92
aPTT (sec)*	28.5 ± 6.9	34.7 ± 8.08
INR*	1.2 ± 0.3	1.3 ± 0.1

ICH, Intracranial Hemorrhage; GCS, Glasgow Coma Scale; aPTT, Activated Partial Thrombin Time; INR, International Normalized Ratio. * $p<0.05$

Table 3. Characteristics of patients with delayed intracranial hemorrhage

Patients	Age	Sex	GCS score	Mechanism of injury	Type of AC/AP drug	Reason for AC/AP treatment	Platelet Count (10^9/L)	INR	Change in Neurological Status	Repeat CT pathologic finding	Neurosurgical Intervention	Outcome
No. 1	79	Male	15	Ground-level fall	Dabigatran	Atrial fibrillation	130	1.2	No	Intraparenchymal hemorrhage	No	Complete recovery
No. 2	70	Male	15	Ground-level fall	Acetylsalicylic acid	Stroke	92	1.4	No	Intraparenchymal hemorrhage	No	Complete recovery
No. 3	87	Male	15	Ground-level fall	Apixaban	Coronary artery disease	267	1.4	No	Intraparenchymal hemorrhage	No	Complete recovery

GCS, Glasgow Coma Scale; AC/AP, Anticoagulant and antiplatelet; INR, International Normalized Ratio.

DISCUSSION

Delayed onset of an intracranial hematoma is a major concern for patients with mild traumatic brain injuries. The use of AC/AP therapy is an independent and major risk factor for intracranial bleeding is. Patients taking AC/AP have been a challenging aspect of mild brain injury management and follow-up. The use of AC/AP drugs increases both early and delayed ICH (ranges from 0 to 6%) after head trauma (5,7,10,15,17,18). Many factors, such as the number and the demographic characteristics of patients included, severity, and mechanism of trauma may affect delayed ICH rates. Menditto et al (7) found a significantly high rate of delayed ICH with 6%. The authors explained that this might probably be due to an INR level greater than 3 in those patients. Additionally, we noticed that the age of the study population was relatively high with a median age of 82 (7).

Studies that aimed to detect delayed ICH, pointed out similar risk factors such as AC/AP drug type, patient age, and INR value. Studies in the past 10 years have mostly focused on patients receiving vitamin K antagonists (VKA), clopidogrel, and ASA (5,6,8,10,19), whereas studies published in the last few years examined mainly the use of direct oral anticoagulant (DOAC) group drugs which use has become more prevalent (20,21). There are a few studies that similarly to us have included a broad range of AC/AP drugs (22). DOACs are known to be safer with an appreciable reduction of spontaneous ICH rate than traditional oral anticoagulants (15,18). However, the outcome after TBI when using a DOAC remains uncertain (23). In our study, two of the 3 patients who revealed delayed ICH at the end of the observation period were using DOAC (respectively dabigatran and apixaban). In their study, Barmparas et al (21) showed that the incidence of delayed ICH was less than 1% in patients on trauma patients under DOACs with initial negative imaging.

Riccardi et al (15) reported a lower incidence of hemorrhage after mild head trauma in patients treated with DOACs compared to VKA. Conversely, a recent study showed a rate smaller than 1% (n=3) for delayed ICH in trauma patients, none of these patients were under DOACs (22). Tauber et al (11) observed that 4% of patients using ASA had a delayed ICH. Swap et al (24) found delayed ICH in 2.7% of the patients who used VKA and in 2.3% of the patients who used clopidogrel. Nishijima et al (8) detected delayed ICH in 0.6% (n=4) of patients using VKA, and none of them was taking clopidogrel. Considering all these results, it can be said that AC/AP drug use alone is not a risk factor for delayed ICH, and this entire drug group may cause delayed ICH.

In our study, the three patients with delayed ICH were over 65 years old. When we looked at the existing literature the majority of patients with delayed ICH were over 65 years old (8,9,16). Age-related cerebral atrophy and posttraumatic hemorrhages association has always been speculation. Likewise, Dunham et al (25) objectively documented that acute post-traumatic ICH and pre-injury cerebral atrophy are clearly correlated.

Many studies reported high INR levels as a risk factor for delayed ICH in patients with head trauma (7,26). In a study conducted by Schoonman et al (10), patients developing delayed ICH, were on VKA (n=5) and three of them had a supratherapeutic level of INR (>3). Patients with delayed ICH may have a normal INR level (defined as between 2-3) (27). Studies have shown that delayed bleeding may occur in patients with head trauma, whose INR value is in the therapeutic range (4,8). In our study, three patients with delayed ICH had a normal range of INR values. Using INR level for risk stratification of brain injury patients on VKA has been suggested however, resulting data should be interpreted rigorously considering the limited number of reported delayed ICH patients in these studies (26,27).

In our study, patients with delayed ICH had no complaints or neurological symptoms during the 24-hour follow-up. Similar to our study, Uccella et al (19) observed no neurological impairment during the observation period. A meta-analysis performed by Verschoof et al (16) showed a low frequency of 0.2% for neurological deterioration by cause of delayed ICH within 24 hours. Furthermore, the majority of patients with delayed ICH is asymptomatic and discharged without surgical treatment; routine observation appears unfounded when the initial head CT is absolutely normal. Hospitalization or follow-up may increase the financial cost of the health system, revealing uncertain benefits (16).

Due to the risk of delayed ICH in head trauma patients using AC/AP drugs, different recommendations have emerged for the management of this patient group. While some studies recommend clinical observation of patients for different periods (4-6,9,19), others affirm that observation will not be sufficient since the majority of patients with delayed bleeding is asymptomatic and a repeat CT before discharge from ED might be beneficial (7,11,28). In a systematic review, development of symptomatic ICH within 24 hours in patients with minor brain injury when the initial head CT was normal is rare, even for patients taking anticoagulants (16). Most of the patients (55% and n=5) with delayed ICH were diagnosed after 24 hours in this review (16). Schoonman et al (10) found that 80% (n=4) of the patients with delayed ICH had bleeding after 24 hours and findings do not support the observation recommendation for at least 24 hours from the European Federation of Neurological Societies. In the study of Afaneh et al (29), only two of 273

patients (0.7%) who underwent a repeat CT had clinically significant delayed ICH but none of the patients had a surgical intervention. Velmahos et al (30) do not recommend routine repeat head CT in patients with minor TBI, suggesting focusing on clinical examination clues to identify the few who will need intervention. Therefore, it can be said that monitoring these patients and undergoing a repeat CT scan may increase the cost burden in healthcare systems and exposure to radiation without providing any significant clinical benefits.

LIMITATIONS

Our study has some limitations for the generalizability of the results. First, our study was conducted in a single hospital and the majority of the patients were elderly and, the most trauma mechanism was ground-level fall so our study may not represent all patient populations. Second, the sample size in our study was relatively low and confidence intervals of the rate of delayed ICH were broad. Third, the number of patients who didn't complete the 24-hour observation and/or a repeat CT scan was high, and these patients may have asymptomatic delayed ICH. Finally, a repeat head CT after 24 hours of admission was not performed in patients who did not use AC/AP drugs therefore we cannot make interpretations on the rate of delayed ICH in this patient group.

CONCLUSION

The rate of delayed ICH after a 24-hour observation in patients with a mild head trauma using AC/AP who had an initially normal head CT is low and a repeat head CT scan may not be necessary. In the light of the literature reviewed, and based on our results, we consider that it would be more appropriate to discharge the mild head trauma patients taking AC/AP medications who had an initially normal head CT and remain clinically stable after a 24-hour observation.

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RESEARCH ARTICLE

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The Effect of Vitamin D Level on the Clinical Situation in COVID-19 Patients

ABSTRACT

Objective: Vitamin D plays an important role in maintaining the integrity of mucosal barriers and in natural and acquired immunity. In the COVID-19 pandemic, the strength of personal immunity is very important in the course of the disease, despite the presence of variants of the virus or vaccination status.

Method: In this study, we investigated the relationship between the clinical course and vitamin D levels of outpatient and inpatient follow-up patients admitted to our hospital due to COVID-19. A total of 94 patients, 47 outpatients and 47 inpatients, were included in the study.

Results: The mean age and gender distributions of both groups were similar. Vitamin D levels were found to be normal in only 7 of 94 patients who were followed up in our hospital due to COVID-19. Patients with vitamin D levels ≥30 were significantly lower than those with "<10" and "10-29.9" ($p<0.01$ for each). Hospitalized patients (71%) with vitamin D levels <10 were significantly higher than those (0%) with vitamin D levels ≥30. Additionally, the outpatients (29%) with vitamin D levels <10 were significantly lower than those (100%) with vitamin D levels ≥30.

Conclusion: The data showed that vitamin D deficiency may be associated with the severe clinical course of COVID-19, even in patients without comorbidities, and may also be one of the predisposing factors resulting in death in COVID-19. As a result, vitamin D levels in COVID-19 patients may be important for the course of the disease.

Keywords: Vitamin D, COVID-19, Clinical Course, Inpatients, Outpatients.

COVID-19 Hastalarında D Vitamini Düzeyinin Klinik Durumla Olan İlişkisi

ÖZET

Amaç: Vitamin D, mukozal bariyerlerin bütünlüğünün korunmasında, doğal ve kazanılmış bağışıklıkta önemli rol oynar. COVID-19 pandemisinde, virüsün varyantlarının varlığına veya aşılanma durumuna rağmen, kişisel bağışıklığın gücü hastalığın seyrinde çok önemlidir.

Gereç ve Yöntem: Bu çalışmada hastanemize COVID-19 nedeniyle başvuran ayaktan ve yatarak tedavi gören hastaların klinik seyri ile D vitamini düzeyleri arasındaki ilişkiyi araştırdık. 47 ayaktan ve 47 yatan hasta olmak üzere toplam 94 hasta çalışmaya dahil edildi.

Bulgular: Her iki grubun ortalama yaş ve cinsiyet dağılımları benzerdi. Hastanemizde COVID-19 nedeniyle takip edilen 94 hastanın sadece 7'sinde D vitamini seviyeleri normal bulundu. D vitamini düzeyi ≥30 olan hastalar, "<10" ve "10-29.9" olanlardan anlamlı derecede düşüktü (her biri için $p<0.01$). D vitamini düzeyi <10 olan hastanede yatan hastalar (%71), D vitamini düzeyi ≥30 olanlardan (%0) anlamlı olarak daha yüksekti. Ek olarak, D vitamini düzeyi <10 olan ayaktan hastalar (%29), D vitamini düzeyi ≥30 olanlardan (%100) anlamlı olarak daha düşüktü.

Sonuç: Veriler, D vitamini eksikliğinin komorbiditesi olmayan hastalarda bile COVID-19'un şiddetli klinik seyri ile ilişkili olabileceğini ve ayrıca COVID-19'da ölümle sonuçlanan predispozan faktörlerden biri olabileceğini gösterdi. Sonuç olarak, COVID-19 hastalarındaki D vitamini seviyeleri hastalığın seyri açısından önemli olabilir.

Anahtar Kelimeler: D Vitamini, COVID-19, Klinik Seyir, Yatan Hastalar, Ayaktan Hastalar.

INTRODUCTION

Severe acute respiratory distress syndrome (ARDS), oxygen desaturation, inflammation, cytokine storm, pneumonia, thrombi/embolism formation and oxidative damage occur as common symptoms in 2019 coronavirus disease (COVID-19) (1). While immune responses specific to COVID-19 are extremely important to eliminate the spread of the virus, uncontrolled inflammatory reactions can trigger systemic damage, especially in the lungs. COVID-19 causes significantly increased morbidity and mortality by causing microvascular thrombosis, oxygen desaturation, differences in lymphocyte and platelet counts and deviations in C-reactive protein and many plasma/serum enzyme levels (2).

Vitamin D is an essential part of the human diet. İt is obtained by skin exposure to sunlight (thereby converting 7-dehydrocholesterol to cholecalciferol, vitamin D3), from foods, or through supplements (3). Vitamin D exists in several forms including 25-hydroxyvitamin D [25(OH)D], the primary circulating form, and 1,25-dihydroxyvitamin D [1,25(OH)2D], the active form (4). Serum 25(OH)D correlates with overall vitamin D stores and is the most commonly used biomarker for assessing vitamin D deficiency. Deficiency is often defined by circulating 25(OH)D levels below 30 ng/ml (75 nmol/l) (5).

Randomized clinical studies have reported effects of vitamin D supplementation in protecting against colds and influenza (6). There are indications of vitamin D being a potent immunomodulator and protective against acute viral respiratory tract infections (7). In recent years, it has been shown that there may be a relationship between COVID-19 infection and vitamin D levels (8). For example, it has been suggested that maintaining optimum levels of vitamin d, thanks to its immunosuppressive effects, may affect the severity of the disease in COVID-19 patients (9). From our literature review, we observed that there are many advantages of prophylactic and therapeutic use of vitamin D in the management of COVID-19 (10).

In this study, we aimed to investigate the effect of vitamin D on clinical status and course in COVID-19 patients.

MATERIAL AND METHODS

Study Design: This study was conducted at Faculty of Medicine in Duzce University between April 01, 2021–May 30, 2021. With the decision number: 2021/157 Clinical Research Ethics Committee at Duzce University approved this study.

We investigated the presence of the Vitamin D's role on the clinical condition in COVID-19 patients. Only the patients admit for COVID to the hospital with positive SARS CoV 2 PCR test (Bio-speedy® SARS CoV-2 RT-qPCR, Turkey) were included in the study. Among the patients included in the study, regardless of COVID-19, they were not receiving vitamin D therapy. In the patients having mild symptoms; normal lymphocyte counts and C reactive protein (CRP) levels and also their oxygen saturation levels were mentioned as in the outpatient group. The patients having severe clinical symptoms were demonstrated among those of whom were hospitalized. In this group, the measurement of lymphocyte counts and O_2 saturation levels were lower than normal. Besides their CRP levels were higher than normal. All cases were divided in two groups as outpatients and inpatients, according to their clinical and laboratory data. Frankly, all those of all patients' blood were obtain on the first and /or third day of their admission to the hospital. The patients' serum samples for measure vitamin D level were stored at -20°C till they were analyzed. Serum vitamin D levels were measured by immunoassay method with Architect 25-OH vitamin D kit (Abbott Diagnostics, Lake Forest, IL, USA). Deficiency is 25(OH)D levels below 30ng/ml (75nmol/l). The patients were divided into 3 groups (<10, 10-29.9, ≥30) according to their vitamin D levels. Considering the detection limits in the laboratory, a vitamin D level of <10 ng/ml indicates low vitamin D levels, a range of 10-29.9 ng/ml is the normal reference value, and a value of ≥30 ng/ml indicates a high level of vitamin D. Lymphocyte counts ≤0.82 ($\times 10^9$/L), mean oxygen saturation (SaO_2) <94, CRP >0.5, ferritin >150ng/ml and D-dimer >0.5 µg/ml were abnormal levels. Presence of diabetes mellitus, hypercholesterolemia/hyperlipidemia, chronic kidney disease, heart failure, coronary and peripheral artery disease were accepted as comorbidity. In addition, CRP (>10), ferritin (>500ng/ml), lymphocyte (<800/µl), D-dimer (>1000ng/ml), oxygen saturation (<93%) and lung involvement by radiological imaging were evaluated as poor prognostic factors.

Radiological Examination: Chest CT images were obtained using a 128-slice multidetector scanner (Somatom definition AS 128, Siemens Healthineers, Erlangen, Germany) with a slice thickness of 1 mm. Both lung (width, 1500 HU; level, -500 HU) and mediastinal (width, 350 HU; level, 40 HU) settings were used in the CT evaluation. Peripheral, bilateral, GGO with or without consolidation or visible interlobular lines (crazy-paving), multifocal GGO of rounded morphology with or without consolidation or visible interlobular lines (crazy-paving) were considered as typical CT findings of COVID-19 on chest CT according to the recommendations of Radiological Society of North America (RSNA) (11).

The presence of multifocal patchy and/or confluent ground glass opacities and consolidations with rounded morphology and coarse horizontal

lines in a bilateral, peripheral and mid to lower zone distribution were considered as highly suggestive findings of COVID-19 on a chest X ray (12).

Statistical Analysis: One Way ANOVA was used for comparison between groups in terms of quantitative variables. Relationships between categorical variables were examined with Pearson Chi-square and Fisher-Freeman-Halton (post hoc: Bonferroni test) tests. Chi-square (post hoc: Bonferroni test) and Fisher Exact tests were used for comparisons between ratios. SPSS 22 program was used for statistical evaluations. $p<0.05$ was considered statistically significant.

RESULTS

The study group consisted of 94 patients, 47 inpatients and 47 outpatients. The mean age was 53.6±15.9 (22-92) years and the number of men and women was equal. Vitamin D levels were <10 ng/ml in 31 patients (33%), 10-29.9 ng/ml in 56 patients (59.6%) and ≥30 ng/ml in 7 patients (7.4%).

Vitamin D levels were found to be normal in only 7 of 94 patients who were followed up in our hospital due to COVID-19. The patients with vitamin D levels ≥30 ng/ml was significantly lower than the patients with vitamin D levels <10 ng/ml and 10-29.9 ng/ml ($p<0.01$ for each).

When the patients were classified according to their vitamin D levels, gender and age distributions were homogeneous ($p=0.100$, $p=0.532$). Inpatient follow-up, high ferritin and D-dimer levels, and poor prognostic factors were found to be significantly higher in patients with low vitamin D levels ($p<0.05$). Apart from these, no significant difference was found between vitamin D levels in terms of radiological involvement, oxygen demand, high CRP, and low lymphocyte count ($p>0.05$). Sociodemographic, clinical characteristics and laboratory results of the patients according to their vitamin D levels were shown in Table 1.

Table 1. Comparison of sociodemographic, clinical and laboratory results of patients according to vitamin D levels

		Experimental groups according to vitamin D levels								p
		<10		10-29.9		≥30		Total		
		n	%	n	%	n	%	n	%	
Gender	**Female**	20	64.5	23	41.1	4	57.1	47	50	0.100
	Male	11	35.5	33	58.9	3	42.9	47	50	
	Total	31	100	56	100	7	100	94	100	
Age*		56.2±17.4 (23-92)		52.1±15.9 (22-86)		53.9±7.5 (44-68)		53.6±15.9 (22-92)		0.532
Clinical status	**Outpatient**	9	29.0	31	55.4	7	100	47	50	**0.001**
	Inpatient	22	71.0	25	44.6	0	0	47	50	
	Total	31	100	56	100	7	100	94	100	
CRP (mg/L)	**0-0.5**	5	16.1	14	25.0	4	57.1	23	24.5	0.074
	>0.5	26	83.9	42	75.0	3	42.9	71	75.5	
	Total	31	100	56	100	7	100	94	100	
Ferritin (ng/ml)	**<20**	1	4.2	2	4.3	1	16.7	4	5.3	**0.003**
	Normal	4	16.7	17	37.0	5	83.3	26	34.2	
	High	19	79.2	27	58.7	0	0	46	60.5	
	Total	24	100	46	100	6	100	76	100	
Lymphocyte count /mm3	**<1000**	17	54.8	30	53.6	3	42.9	50	53.2	0.938
	1000-3700	14	45.2	25	44.6	4	57.1	43	45.7	
	>3700	0	0	1	1.8	0	0	1	1.1	
	Total	31	100	56	100	7	100	94	100	
D-dimer (µg/mL)	**0-0.5**	9	30	31	56.4	6	100	46	50.5	**0.002**
	>0.5	21	70	24	43.6	0	0	45	49.5	
	Total	30	100	55	100	6	100	91	100	
Radiological involvement	**Yes**	21	67.7	40	71.4	2	28.6	63	67.0	0.087
	No	10	32.3	16	28.6	5	71.4	31	33.0	
	Total	31	100	56	100	7	100	94	100	
Oxygen demand	**Yes**	14	45.2	18	32.1	0	0	32	34.0	0.060
	No	17	54.8	38	67.9	7	100	62	66.0	
	Total	31	100	56	100	7	100	94	100	
Presence of at least one of the poor prognostic factors	**Yes**	21	67.7	31	55.4	1	14.3	53	56.4	**0.036**
	No	10	32.3	25	44.6	6	85.7	41	43.6	
	Total	31	100	56	100	7	100	94	100	

*mean±standard deviation (minimum-maximum)

While 22 of the patients (71%) with vitamin D levels <10 ng/ml and 25 of the patients (44.6%) with vitamin D levels between 10-29.9 ng/ml were hospitalized, there was no inpatient treatment among the patients with vitamin D levels ≥30 ng/ml. In Figure 1, the clinical situation of the patients according to their vitamin D levels were indicated.

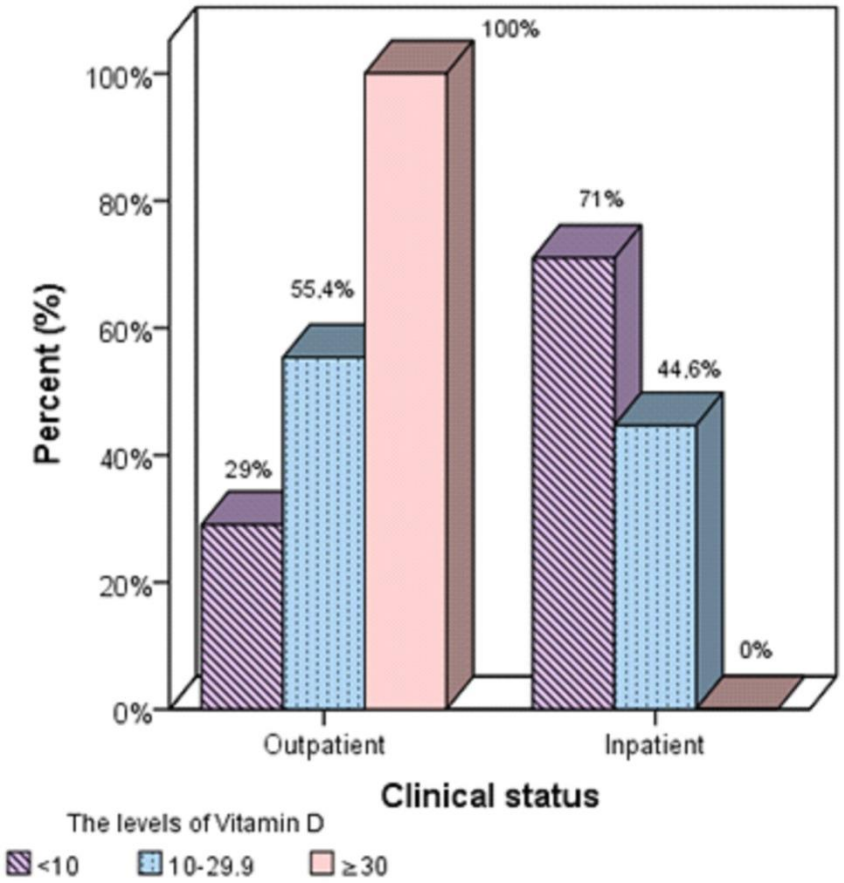

Figure 1. Distribution of clinical situations according to vitamin D levels

The proportion of inpatient (71%) with vitamin D levels <10 ng/ml was significantly higher than that of patients (0%) with vitamin D levels ≥30 ng/ml. In addition, the proportion of outpatients (29%) with vitamin D levels <10 ng/ml was significantly lower than that of those (100%) with vitamin D levels ≥30 ng/ml. The proportions of patients with poor prognosis markers with vitamin D levels <10 ng/ml (67.7%) and 10-29.9 ng/ml (55.4%) were significantly higher than those with vitamin D levels ≥30 ng/ml (14.3%) ($p<0.05$).

The distribution of the patients in terms of the presence of co-morbidity according to their clinical status was given in Table 2. There was a significant difference in the presence of co-morbidity according to clinical status ($p<0.05$). The incidence of co-morbidity in inpatients was 63.8% (n=30), while it was 34% (n=16) in outpatients. Accordingly, the rate of co-morbidity in inpatients was significantly higher than in outpatients ($p<0.05$).

Table 2. The presence of co-morbidity and the effect of vitamin D levels on clinical status

Presence of co-morbidity	25(OH) Vitamin D level (ng/ml)	Clinical status						p
		Outpatient		Inpatient		Total		
		n	%	n	%	n	%	
Yes	**<10**	4	25.0	18	60	22	47.8	**0.039**
	10-29.9	11	68.8	12	40	23	50	
	≥30	1	6.3	0	0	1	2.2	
	Total	16	100	30	100	46	100	
No	**<10**	5	16.1	4	23.5	9	18.8	0.153
	10-29.9	20	64.5	13	76.5	33	68.8	
	≥30	6	19.4	0	0	6	12.5	
	Total	31	100	17	100	48	100	

The presence of co-morbidity, vitamin D levels, and distribution of clinical status of the patients were presented in Table 3. While a significant difference was found in vitamin D levels in patients with co-morbidity according to clinical status ($p<0.05$), it was not observed in patients without comorbidity ($p>0.05$). While vitamin D levels were <10 ng/ml in 60% of the inpatients with co-morbidity, the rate of outpatients was 25%, and the rate of inpatients with related characteristics was significantly higher ($p<0.05$).

Table 3. Comparison of disease outcome, presence of co-morbidity and vitamin D levels

				Groups according to vitamin D levels								p
				<10 ng/ml		10-29.9 ng/ml		≥30 ng/ml		Total		
				n	%	n	%	n	%	n	%	
Co-morbidity	Yes	Disease outcome	Death	8	36.4	2	8.7	0	0.0	10	21.7	0.057
			Healing	14	63.6	21	91.3	1	100	36	78.3	
			Total	22	100	23	100	1	100	46	100	
	No		Death	0	0.0	2	6.1	0	0.0	2	4.2	0.999
			Healing	9	100	31	93.9	6	100	46	95.8	
			Total	9	100	33	100	6	100	48	100	

Vitamin D levels were <10 ng/ml in 4 (23.5%) of 17 inpatients and without co-morbidity, and 10-29.9 ng/ml in 13 (76.5%). Accordingly, vitamin D levels were deficient or insufficient in all inpatients who did not have any co-morbidities.

Of 94 patients, 82 (87%) were cured and 12 (13%) died. Eight (66.7%) of the 12 patients who died had vitamin D levels <10 ng/ml, while 4 (33.3%) had a vitamin D levels 10-29.9 ng/ml. There was no death in patients with a vitamin D levels ≥30 ng/ml. There was no difference between the rates of those with vitamin D levels <10 ng/ml and those with 10-29.9 ng/ml levels ($p=0.248$). Two of the patients who died without co-morbidities had vitamin D deficiency.

DISCUSSION

Vitamin D, which has been thought to be related to bone health for many years, has been accepted as a vitamin and even a hormone that is effective in many diseases such as immune system, cell renewal, course of infections, allergies, autoimmune diseases. Although there are many reasons for this, the most basic of them is that people spend less time outdoors and use high protection factor creams while sunbathing. In addition, another reason is that vitamin D is low in animal foods, which are our main source of vitamin D. This is because animals are mostly kept in closed areas instead of pastures (13). People also consume meat, eggs and dairy products that are low in vitamin D from these sunlight-deprived animals. For these reasons, vitamin D deficiency has become common in societies.

In recent years, there is evidence that vitamin D deficiency is closely related to the severity of infections. It has been reported that vitamin D has positive effects on the strength of physical barriers, which are the foundations of infection immunity, and on the development of natural and acquired immune response (14). Many studies have been conducted which proved that over time Vitamin D did show improvement in the survival rate. Vitamin D has many mechanisms by which it reduces the risk of microbial infection and death (15). A recent review regarding the role of vitamin D in reducing the risk of the common cold grouped those mechanisms into three categories: physical barrier, cellular natural immunity, and adaptive immunity (16). In this study, we determined the effect of vitamin d deficiency on the severity of the clinical condition in COVID-19 patients. In our study, only 7 of the inpatient and outpatient COVID-19 patients had normal vitamin D levels. All inpatients had vitamin D deficiency/insufficiency. Vitamin D levels were low in all patients who died and were inpatient, with or without co-morbidity. To our best knowledge, COVID-19 is more serious in people with co-morbidities. In our study, we found that all inpatients had vitamin D deficiency/insufficiency, although they did not have co-morbidity.

Severe COVID-19 is characterized by an over-response of the immune system called as a cytokine storm. Infection is largely limited by the strength of the mucosal barriers and innate immune response in individuals with normal vitamin D levels (17). Moreover, serious infections do not develop in these people. Several studies demonstrated the role of vitamin D in reducing the risk of acute viral respiratory tract infections and pneumonia (18). Additionally, it was reported that high levels of vitamin D can reduce pulmonary fibrosis by reducing interleukin 1 beta levels of pro-inflammatory cytokines produced by pulmonary fibroblast cells in a mouse model of bleomycin-induced lung fibrosis (19). These include direct inhibition with viral replication or with anti-inflammatory or immunomodulatory ways. Petrelli and colleagues have associated the risk of COVID-19 infection with patients with low vitamin D levels resulting in a worse prognosis and higher mortality rate compared to patients with vitamin D levels in the normal range (20). Therefore, vitamin D levels might be associated with the course of COVID-19 disease.

CONCLUSIONS

In line with our findings, vitamin D deficiency is associated with severe COVID-19. Many similar studies have been found in the literature. It is very important for people to keep their vitamin D levels within normal levels by using vitamin D before they get sick.

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RESEARCH ARTICLE

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Morphological and Morphometric Analysis of Hypoglossal Canal and Its Importance in Cranial Base Surgery: A Skull Study

ABSTRACT

Objective: This study aims to analyze the morphological and morphometric features of the hypoglossal canal (HC) and its topographical relationship with the occipital condyle (OC) and to present guiding information to the relevant field experts.

Method: This research was carried out with 33 adult Turkish skulls of unknown gender. Typing definitions of the skulls without damage to the posterior cranial fossa were made with the parameters related to a standard caliper and a protractor.

Results: The distance of the extracranial opening of the HC to the posterior end of the OC was 14.37±1.76 mm on the right side and 16.37±1.76 mm on the left ($p<0.05$), while the intracranial opening was determined to be greater than that of the OC and its distance to the posterior end was calculated as 8.95±1.00 mm on the right side and 10.41±1.24 mm on the left side ($p<0.05$). The angle between the extracranial opening of the HC/pharyngeal tubercle was 45.97±2.06 degrees on the right side and 48.05±2.97 degrees on the left side ($p<0.05$). It was determined that Type I was observed most frequently on the right side with a rate of 42.42% and on the left side with a rate of 18.18%. Bilaterally, only Type I and Type II b were detected.

Conclusion: We believe that our research will guide surgeons, anatomists, and anthropologists in terms of making transcondylar, supracondylar, and lateral suboccipital approaches and examining the canal differences in Turkish society.

Keywords: Supracondylar Approach, Hypoglossal Canal, Occipital Condyle, Osteotic Variations.

Canalis Nervi Hypoglossi'nin Morfolojik/Morfometrik Analizi ve Kraniyal Taban Ameliyatlarında Önemi: Kuru Kafatası Çalışması

ÖZET

Amaç: Bu çalışmanın amacı canalis nervi hypoglossi'nin morfolojik ve morfometrik özelliklerini ve condylus occipitalis ile topografik ilişkisini analiz ederek ilgili alan uzmanlarına yön gösterici bilgileri sunmaktır.

Gereç ve Yöntem: Bu araştırma cinsiyeti bilinmeyen 33 yetişkin Türk kafatası ile yapılmıştır. Fossa cranii posterior hasarı olmayan kafataslarının standart kumpas ve bir açı ölçer ile ölçülerek ilgili parametreler ile tiplendirme tanımlamaları yapıldı.

Bulgular: Canalis nervi hypoglossi'nin ekstrakraniyal açıklığının condylus occipitalis'in arka ucuna olan mesafesi sağ tarafta 14,37±1,76 mm, sol tarafta ise 16,37±1,76 mm ($p<0,05$) olarak belirlenirken intrakraniyal açıklığın condylus occipitalis'in arka ucuna olan mesafesi ise sağ tarafta 8,95±1,00 mm, sol tarafta 10,41±1,24 mm ($p<0,05$) olarak hesaplanmıştır. Tuberculum pharyngeum-canalis nervi hypoglossi'nin ektrakraniyal açıklığı arasındaki açının sağ tarafta 45,97±2,06 derece, sol tarafta ise 48,05±2,97 derece olduğu belirlenmiştir ($p<0,05$). En fazla Tip I'in sağ tarafta %42,42, sol tarafta ise %18,18 oranında gözlendiği belirlendi. Bilateral olarak sadece Tip I ile Tip II b tespit edilmiştir.

Sonuç: Canalis nervi hypoglossi'nin osteotik varyasyonları, içinden geçen hassas nörovasküler yapılar açısından klinik olarak önemlidir. Literatürde yer alan farklı ırklardaki dış açıklığın canalis nervi hypoglossi'nin midsagittal düzleme olan sağ ve sol eğim açısı daha fazla iken araştırmamızda Türk toplumuna ait kafataslarındaki bu açıların daha az olduğu ve canalis nervi hypoglossi ile condylus occipitalis arasındaki mesafelerin diğer popülasyonlara oranla daha kısa olduğu tespit edilmiştir. Dolayısı ile araştırmamızın transkondiler, suprakondiler ve lateral suboksipital yaklaşım yapmak ve Türk toplumuna ait kanal farklılıklarını incelemek açısından cerrahlar, anatomistler ve antropologlara yol gösterici olacağı kanaatindeyiz.

Anahtar Kelimeler: Suprakondiler Yaklaşım, Canalis Nervi Hypoglossi, Condylus Occipitalis, Osteotik Varyasyonlar

INTRODUCTION

Hypoglossal canal (HC), is a narrow canal located in the anterolateral of the foramen magnum (FM), in front of the occipital condyle (OC) and inferomedial of the jugular foramen, through which the meningeal branch of the pharyngeal artery, small vein plexuses and hypoglossal nerve pass (1-3). Since HC is adjacent to the occipital lobe, cerebellum and brain stem in terms of location and morphology, it has the feature of a land-marker for surgical intervention in skull base variations and pathologies in this region (tumors, aneurysms, congenital or acquired malformations and trauma). Therefore, it is clinically very important to know the anatomy of the HC located in the posterior cranial fossa in order to determine the appropriate surgical approach (4,5).

Lesions of HC are usually benign and rare. They include hypoglossal posterior cranial fossa meningiomas, schwannomas, and jugular-tympanic paragangliomas. The outcome of surgically curing these lesions is linked to the size and type of tumor and the anatomical variations of HC. Therefore, knowing the morphometric details of HC and its relationship with neighboring structures provides wider access and a better surgical angle for safe and successful surgery (6). The distant lateral transcondylar approach and its modifications require extensive vertebral artery dissection, HC exposure, and removal of OCs and the jugular tubercle. Random manipulations during condylectomy may damage the jugular vein, vertebral artery and bulb (6), also may cause craniocervical instability by damaging the lower cranial nerves (4). In addition, the supracondylar approach, which is part of the distant lateral transcondylar approach, provides access to the medial area of the HC and the jugular tubercle. During this procedure, the meningeal branches of ascending pharyngeal artery and the hypoglossal nerve in the HC may be damaged (7). It is important to know the anatomy of this region in order to prevent damage during the surgical intervention (6-15).

When the literature was examined; it has been observed that there is not enough data on osteotic variations in HC and its relationship with PT and OC in Turkish population. Therefore, the aim of this study is to analyze the morphological and morphometric features of HC. In briefly, knowing the morphometry of this region well can provide a safe surgical procedure for supracondylar, transcondylar and lateral suboccipital approaches. Preoperative morphometric analysis of HC will support the surgeon in selecting the most suitable surgical practice and approach to be used.

MATERIAL AND METHODS

Study Design: This research was carried out with 33 adult craniums of unknown gender found in the Anatomy laboratories of Ankara Medipol University and Erciyes University Faculty of Medicine. Approval was obtained from the Ethics Committee of Ankara Medipol University Faculty of Medicine in order to conduct the study (19.09.2022/161).

Inclusion Criteria: Skulls with preserved bone integrity and no structural defect were included in the study.

Exclusion Criteria: Bones with fractured right-left hypoglossal canal were excluded from the study.

Morphological Measurements: Measurements were made by selecting craniums that were not damaged in the posterior cranial fossa. All measurements were performed using Berin's protocols of Kumar et al. (16), Muthukumar et al. (17), and Naderi et al. (18)

The Determined Parameters on the External Skull Base:

• The distance of the extracranial opening of the HC from the posterior end of the occipital condyle (OC)
• Distance of the intracranial opening of the HC from the posterior end of the OC
• Distance between HC-intracranial opening - Opisthion (mm)
• Distance between HC-extracranial opening - Opisthion (mm)
• Distance between HC-intracranial opening - Basion (mm)
• Distance between HC-extracranial opening - Basion (mm)
• Distance of HC from the anterior edge of the intracranial opening to the OC (mm)
• Diameter of the intracranial opening of the HC (mm^2)
• Diameter of the extracranial opening of the HC (mm^2)
• Length of the hypoglossal canal
• The distance of the HC from the intracranial opening to the jugular foramen (JF)
• The distance of the extracranial opening of the HC to the JF (mm)
• Angle of inclination of HC to the midsagittal plane (in degree)*
• Angle between pharyngeal tubercle and extracranial opening of HC (in degree)*
• Angle where two HCs intersect each other in the posterior (in degree)*
• Angle between the pharyngeal tubercle and the extracranial openings of two opposite HCs (in degree)*

In addition, the morphological variability of HC due to the presence of spurs, incomplete and complete septa was examined in the study. These morphological variations of HC were defined on the axis of typing described by Kumar et al. (16)

Classification of morphological variations in the HC into different types

***Type I** - No evidence of bony spur or septum
***Type II** - Spur HC

A. Spur present near external opening of HC
B. Spur present near internal opening of HC
C. Spur present in the middle part of HC

***Type III** - Incomplete septa dividing a portion of HC into two parts

A. Septa present near external opening dividing it into double external opening
B. Septa present near internal opening dividing it into double internal opening
C. Septa present in the middle part dividing a portion of HC into two

***Type IV** - Complete septa dividing whole HC into two parts

Statistical Analysis: The statistical analysis was performed using SPSS version 22.00. Right and left sides were investigated with student's t-test. The relationship between the variables was analyzed with the pearson's correlation test. A significance level of was assumed as $p<0.05$.

RESULTS

Morphological measurements of HC are given in Table 1. The mean distance of the extracranial opening of the HC to the posterior end of the OC was 14.37±1.76 mm on the right side and 16.37±1.76 mm on the left side. The mean distance of the intracranial opening to the posterior end of the OC was determined as 8.95±1.00 mm on the right side and 10.41±1.24 mm on the left side. It was observed that both measurements were statistically significantly higher on the left side. Measurements related to the extracranial and intracranial openings of HC are given in Table 1 and statistical significance was not found between the right and left sides.

Table 1. Morphological measurements of HC

	Right (mm)		**Left (mm)**		***p***
	Range	**Mean**	**Range**	**Mean**	
The distance of the extracranial opening of the HC from the posterior end of OC (mm)	11.9-18.9	14.37±1.76	9.14-18.21	16.37±1.76	**.021**
Distance of the intracranial opening of the HC from the posterior end of the OC (mm)	7.01-11.10	8.95±1.00	7.41-12.13	10.41±1.24	**.041**
Distance between HC-intracranial opening - Opisthion (mm)	9.72-37.24	29.42±4.81	20.22-34.78	29.61±3.01	.420
Distance between HC-extracranial opening-Opisthion (mm)	23.41-43.02	38.70±3.57	22.43-43.37	39.05±3.78	.797
Distance between HC-intracranial opening-basion (mm)	9.29-17.49	12.83±1.87	9.87-17.39	12.88±1.68	.471
Distance between HC-extracranial opening - basion (mm)	14.48-40.21	17.52±4.33	13.85-42.33	17.96±4.65	.915
Distance of HC from the anterior edge of the intracranial opening to the OC (mm)	6.66-12.32	9.28±1.31	7.02-77.81	11.86±11.94	.125
Diameter of the intracranial opening of the HC (mm^2)	1.15-7.29	4.68±1.27	1.23-2.95	4.35±2.12	.238
Diameter of the extracranial opening of the HC (mm^2)	1.67-5.81	3.78±1.17	1.37-8.15	4.17±1.51	.695
Length of hypoglossal canal (mm)	5.38-15.03	11.24±2.24	6.00-14.51	11.11±2.09	.700
The distance of the HC from the intracranial opening to the JF (mm)	4.13-12.31	7.85±1.90	1.68±14.38	8.12±2.77	.342
The distance of the extracranial opening of the HC to the JF (mm)	7.21-15.23	10.86±2.04	7.99-13.25	10.82±1.43	.061

HC: Hypoglossal canal, OC: Occipital condyle, JF: Jugular foramen

The distance between the right and left hypoglossal canal was determined as 24.39±4.93 mm from the inner rim and 32.25±2.48 mm from the outer rim, and it was determined that there was statistical significance between the inner and outer rims. HC showed an angulation of 26.64±2.97 degrees on the right and 26.03±2.97 degrees on the left in the midsagittal plane; The angle formed when two opposing HCs merged in the pharyngeal tubercle was found to be 84.90±6.89 degrees (Table 2). It was determined that the angle between the extracranial opening of the pharyngeal tubercle (TP)-HC was 45.97±2.06 degrees on the right side and 48.05±2.97 degrees on the left side, and the difference was statistically significant (*$p<0.05$*, Table 2, Figure 1).

Table 2. Angular measurements of HC

	Right	Left	*p*
Angle of inclination of HC to midsagittal plane (in degree)*	26.64±2.97	26.03±2.97	0.53
Angle between pharyngeal tubercle and extracranial opening of HC (in degree)*	45.97±2.06	48.05±2.97	**0.043**
Angle where two HCs intersect each other in the posterior (in degree)*		51.04±4.28	
Angle between the pharyngeal tubercle and the extracranial orifices of two opposite HCs (in degree)*		84.90±6.89	

HC: Hypoglossal canal

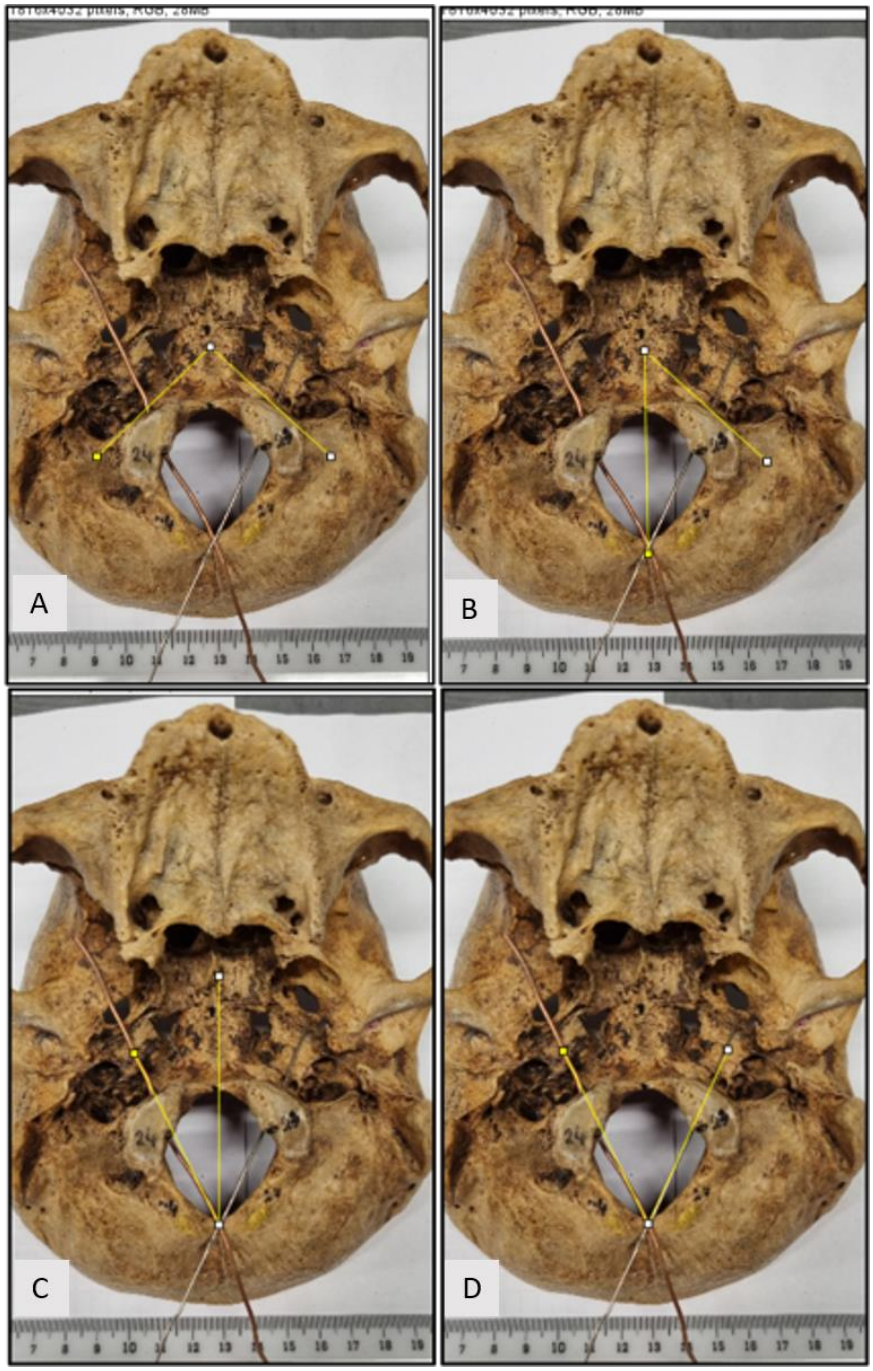

Figure 1. A: Pharyngeal tubercle-angle between the extracranial openings of two opposite HCs, B: Angle between pharyngeal tubercle-extracranial openings of HC C: Angle of inclination of HC to midsagittal plane (in degree), D: Angle where two HCs intersect each other in the posterior

The incidence rates of variations of HC are given in Table 3. Accordingly, it was determined that Type I was the most common, with a rate of 42.42% on the right side and 18.18% on the left side. It was determined that Type II B, which is the second most common type, was observed with a rate of 12.12% on the right side and 21.21% on the left side. Bilaterally, only Type I and Type II b were detected (Table 3, Figure 2,3).

Table 3. Morphological parameters of HC- Frequency of osteotic variations in HC and its distribution in 33 dry skulls.

Side	Type		Right		Left	
			N	%	N	%
Unilateral	Tip I		14	42.42	6	18.18
	Tip II	A	1	3.03	1	3.03
		B	4	12.12	7	21.21
		C		-	2	6.06
	Tip III	A	1	3.03	1	3.03
		B	1	3.03	5	15.15
		C	1	3.03	1	3.03
	Tip IV		1	3.03		-
Bilateral	Tip I		8	24.24	8	24.24
	Tip II B		2	6.06	2	6.06
	Toplam		33	100	33	100

* Significant $p < 0.05$. **in case of double internal opening of HC (type 3). anterior opening was considered along with the single internal opening of the HC (Type1 and Type 2). *** in case of double internal opening of HC (type 3). posterior opening was considered
HC: Hypoglossal canal

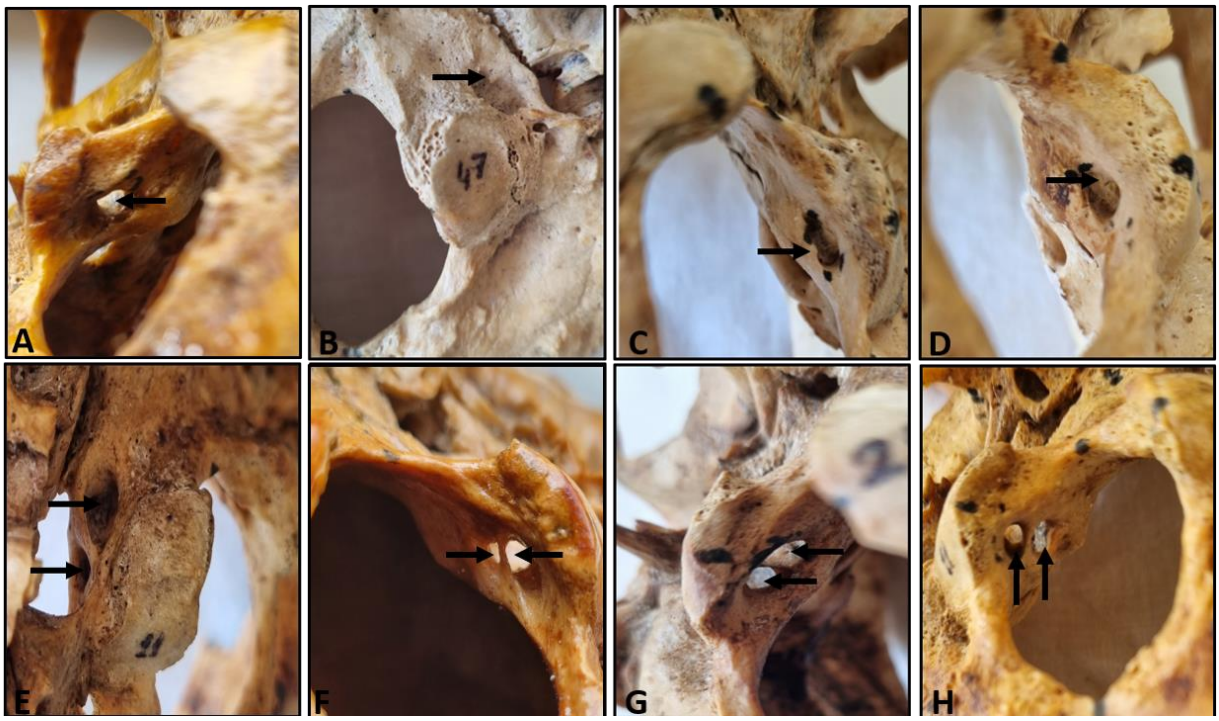

Figure 2. Image of hypoglossal canal variations A: TypeI, B: TypeIIA, C: TypeIIB, D: TypeIIC, E: TypeIIIA, F: TypeIIIB, G: TypeIIIC, H: TypeIV

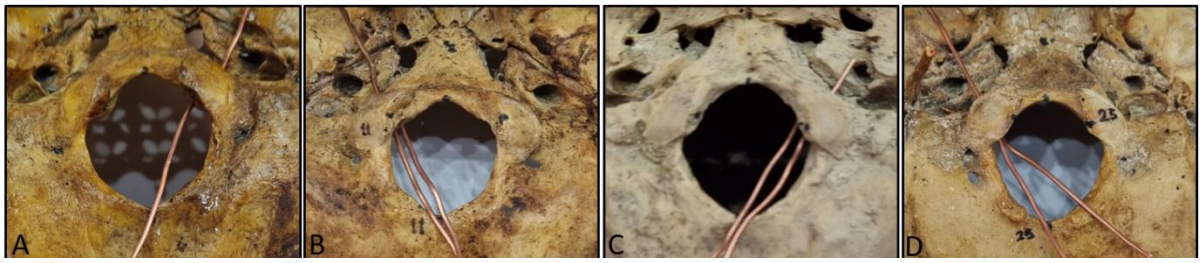

Figure 3. Canal view according to internal and external canal openings A: Single canal opening in both sections, B: Two canal openings in the internal section and one canal opening in the external section for the right hypoglossal canal, C: Two canal openings in the internal and external section for the left hypoglossal canal one duct opening, D: Two duct openings in both sections

DISCUSSION

To understand the skull base morphology in detail and to know the regional variations is a prerequisite for surgical interventions to the skull base, especially the distant lateral approach (11,19,20). It has been reported in many studies that high mortality and morbidity are observed in skull base surgery when the surgical procedure is started without detailed morphological analysis (21,22). Therefore, defining the localization of HC is extremely important both for its proximity to environment structures and for remove of tumors located near or within the canal (23-26). Therefore, we think that the current anatomy should be evaluated by making a detailed analysis in order to improve surgical techniques and facilitate safe surgical to the cranial base. This research was conducted to investigate the morphometric and morphological analysis of HC from an anatomical and clinical perspective.

In this study, the mean length of the HC was 11.24±2.24 mm on the right side and 11.11±2.09 mm on the left side, while the mean intracranial diameter of the canal was 4.68±1.27 mm^2 on the right side and 4,35±2.12 mm^2 on the left side. The mean extracranial diameter was calculated as 3.78±1.17 mm^2 on the right side and 4.17±1.51 mm^2 on the left side. In a study conducted with the Greek population, the intra and extracranial diameters were 5.93 mm^2 and 5.03 mm^2; respectively (26). In another study conducted on Americans and Egyptians, the intracranial diameter was calculated as 5.65 mm^2 and the extracranial diameter was calculated as 5.15 mm^2 (Table 4) (22).

Table 4. Comparison of the incidence of spur and septa. length. Internal/external diameter and in the HC with previous studies.

Researchers	Year	Population	HC length	Extracranial Diameter (mm^2)	Intracranial Diameter (mm^2)	Spur	Incomplete septa		Complete septa
							Intracranial opening	Extracranial opening	
Our study	2022	Turkish	11.18	3.98	4.52	%22.73	%9.1	%3.03	%1.52
Kumar et al.	2017	North Indian	-	-	-	%28	%26	%4	-
Peraskavas et al.	2009	Greek	9.58	5.03	5.93	%18.9	%19.8	-	%1.7
Katsuta T et al.	2000	US and Indian	8.95	5.15	5.65	%10	-	-	-
Bastianini A et al.	1985	Sienese	-	-	-	%35	-	-	-

HC: Hypoglossal canal

The fact that the canal diameter of the Turkish population was found to be smaller in our study compared to other populations suggests that surgeons should pay attention to the diameters of the openings when determining the range of motion in tumor removal operations.

Tumors such as jugulo-tympanic paragangliomas, posterior cranial fossa meningiomas, hypoglossal nerve schwannomas and are frequently seen in HC (24). Vascular lesions of the vertebral artery, dural tumors and congenital lesions are among the commonly encountered lesions around the FM and the caudal surface of the superior cervical spine, lower clivus, which are closely related to HC. Therefore, any osteotic variation in HC will further complicate the pathological conditions mentioned and force surgeons to increase their awareness of these variations (25). Osteotic variations of HC are clinically important for the delicate neurovascular structures that pass through them. The spurs and partitions in the channel will divide the channel into compartments that can compress these structures. In our study, both spur and septa were more common on the left side, while Paraskevas GK et al. (26) observed more frequent variation on the right side in the Greek population. While the incidence of spurs was reported as 18.96% by Paraskevas et al., 35% by Bastianini et al. and 28% by Kumar et al., it was determined as 22.73% in our study.

In the study of Kumar et al., it was observed that incomplete septa formation was more common in the HC internal aperture (26%) than in the external aperture (4%) of the North Indian population (16). Similarly, in our study, it was found that the incidence of septa formation in the inner opening of the canal (9.1%) was higher than the external opening (3.03%). In addition, spur formation was observed in the bilateral internal opening (6.06%) in 2 skulls, while no spur or septa formation was observed bilaterally in either the internal or external opening (24.24%) in 8 skulls. This situation guides surgeons against nerve damage regarding whether the structures passing through the canal, especially the hypoglossal nerve, show division during the intervention to the canal.

While completed septa formation was observed in the Greek population at a rate of 1.7% in the study of Paraskevas et al. (26), it was detected at a rate of 1.52% in our study and was observed to separate the right-sided HC into two channels. Although it is a rare condition; the presence of dividing channels can be encountered in Turkish and Greek populations.

In addition, when drilling OC, the operating needs to estimate the probable bottom and aspect of the HC.[28] In this study, the mean distance of the posterior end of the OC to the outer and inner openings of the HC was 14.37±1.76 and 8.95±1.00 mm on the right side, and 16.37±1.76 and 10 on the

left side, respectively. Similar to our study, Kumar et al., Muthukumar et al., Kızılkanat et al. and Parvindokht et al. declared that the distance of HC to the posterior edge of the OC varies between 11.42-12.3 mm (16,17,23,27). While the measurements in these studies were higher than our measurements, the results of Wen et al. (6) were lower than our results. According to the results of studies on the OC and HC relationship that differs between populations, a wider area can be used during the supracondylar approach to the canal in Northern and Southern Egyptian and Iranian populations, while a narrower intervention area should be used in the Turkish population.

In our study, the right inclination angle of the external opening of the HC to the midsagittal plane was found to be higher than the left inclination angle. The tilt angle indicated by Muthukumar et al. (17) and Paraskevas et al. (26) was higher than in our study. Accordingly, the angle of inclination to be calculated for the far lateral approach in the Turkish population should be made more parallel and closer to the midline compared to other populations.

PT is the central point of the nasopharynx also is important for both breathing and swallowing together with the adjacent anatomical structures. In spite of the significance of this area, the deficiency of the number of evaluations and the lack of research on its relationship with HC required us to survey this region in elaboration morphometric (11). In our study, we determined the angle between the pharyngeal tubercle and the extracranial opening of the HC as 45.97±2.06° on the right, 48.05±2.97° on the left, and the angle between the PT and the extracranial openings of the two opposite HCs as 84.90±6°. In the surgery of this region, it should be kept in mind that the hypoglossal nerve may be within an angle of 45-50° when intervening from the midline, and within an angle of 80-85° in lateral approaches. In conclusion, in skull base surgery performed in the pharyngeal tubercle and craniocervical region tumors, attention should be paid to the position of the external opening of the HC and its angulation to the midline, and the difference in angulation on the right and left sides should be considered.

CONCLUSION

In studies of different populations in the literature, the left inclination angle of the external aperture HC to the midsagittal plane is higher than the right inclination angle, whereas in our study, these angles in the skulls of Turkish populations are less and the distances between HC and OC are shorter than in other populations. Therefore, we believe that our study will guide surgeons who perform transcondylar, supracondylar and lateral suboccipital approach surgeries, as well as anatomists and anthropologists in terms of examining the canal differences of Turkish society.

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RESEARCH ARTICLE

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Is It Time to Add Domains of Quality Of Life to the Childhood Asthma Control Test and the GINA Criteria?

ABSTRACT

Objective: To measure the quality of life by using the Standardized Pediatric Asthma Quality of Life Questionnaire (PAQLQ(S)) in children with asthma and to determine the association of the Childhood Asthma Control Test(c-ACT) and Global Initiative for Asthma(GINA) criteria of asthma control with the PAQLQ(S) domains.

Method: This study was planned in a cross-sectional design. Parents of children between the ages of 7-11 who had been followed up at least ≥1 year with a diagnosis of asthma according to GINA in the Pediatric Allergy and Immunology Clinics of Mersin City and Adana City Research and Training Hospital were invited to participate in the study. Children who fulfilled inclusion criteria enrolled consecutively as they were administered to the clinics in the study period from January to June. Children filled out PAQLQ(S). The levels of asthma control were defined by c-ACT and GINA criteria of asthma control.

Results: Of the total 120 children, the median (%25-75) PAQLQ(S) and c-ACT scores were 4.90(1.3) and 17.9(4.8). All children had quality of life impairments except four. According to c-ACT, 41.7% of children were controlled; 58.3% of them were uncontrolled. PAQLQ(S) scores were found to be correlated with both c-ACT scores ($p<0.001$, $r=0.612$). The c-ACT score was more significantly correlated with the symptom domain of PAQLQ(S)($r=0,667$, $p<0,001$). A more significant positive correlation was found between c-ACT and the duet score of activity and emotional function ($r=0.930$; $p<0.001$).

Conclusion: Asthma affects the quality of life in children. PAQLQ(S) scores decrease significantly when asthma is out of control. PAQLQ(S) is more significantly associated with c-ACT than GINA criteria. It would be useful in clinical practice if c-ACT or GINA criteria can be improved to cover quality of life, at least the part of the quality of life related to the symptoms or the activity limitations and emotional functions.

Keywords: Asthma Control, Asthma Control Test, Childhood's Asthma, Quality of Life.

Çocukluk Astım Kontrol Testi ve GINA Kriterlerine, Yaşam Kalitesi Alanlarını Eklemenin Zamanı Geldi mi?

ÖZET

Amaç: Astımlı çocuklarda Standart Pediatrik Astım Yaşam Kalitesi Anketi (PAQLQ(S)) kullanılarak yaşam kalitesini ölçmek; Çocukluk Çağı Astım Kontrol Testi (c-ACT) ile Global Initiative for Asthma (GINA) Testinin PAQLQ(S) alanları ile ilişkisini belirlemektir.

Gereç ve Yöntem: Bu çalışma kesitsel olarak planlandı. Mersin ve Adana Şehir Hastanesi Pediatrik Alerji ve İmmünoloji Kliniklerinde ≥1 yıldır astım tanısı ile ile izlenen 7-11 yaş arası tüm çocukların evebeynleri çalışmaya davet edildi. Çalışma kriterlerini karşılayan çocuklara polikliniklere çalışma süresi Ocak-Haziran boyunca, polikliniğe başvurdukları sıraya göre ardosıra alındı. Çocuklar PAQLQ(S)'yi doldurdu. Astım kontrol seviyeleri, c-ACT ve GINA kriterleri ile aracılığıyla tanımlandı.

Bulgular: Toplam 120 çocuğun medyan(%25-75) PAQLQ(S) ve c-ACT puanları 4.90(1.3) ve 17.9(4.8) idi. Dördü dışında tüm çocukların yaşam kalitesinde bozulma vardı. c-ACT'ye göre çocukların %41,7'si kontrol altındaydı. PAQLQ(S) skorları her iki c-ACT skoru ile korele bulundu($p<0.001$, $r=0.612$). c-ACT skoru, PAQLQ(S)'nin semptom alanı ile daha anlamlı bir şekilde koreleydi ($r=0,667$, $p<0,001$). c-ACT ile aktivite ve emosyonel fonksiyon düet skoru arasında daha anlamlı bir pozitif korelasyon bulundu ($r=0.930$; $p<0.001$).

Sonuç: Astım çocuklarda yaşam kalitesini etkilemektedir. PAQLQ(S) puanları, astım kontrolden çıktığında önemli ölçüde düşer. PAQLQ(S), GINA kriterlerine kıyasla c-ACT ile daha anlamlı bir şekilde ilişkilidir. c-ACT veya GINA kriterleri, yaşam kalitesini kapsayacak şekilde iyileştirilmesi klinik uygulamada yararlı olacaktır.

Anahtar Kelimeler: Astım Kontrolü, Astım Kontrol Testi, Çocukluk Çağı Astımı, Yaşam Kalitesi

INTRODUCTION

Asthma is one of the most common chronic diseases in children (1). The disease does not just place a burden on health care systems, but it also places a burden on people's social lives; causes physical, educational and emotional impairments(1,2). The asthma treatment's aim is to achieve and maintain clinical control and reduce future risk of adverse outcomes. Clinical control of asthma is assessed by symptoms and lung function measurements according to Global Initiative for Asthma (GINA) guideline(3). A validated and simple method for measuring asthma control in daily practice is to use composite control measures such as asthma control test, childhood asthma control test, and asthma control questionnaire(3,5).

Quality of life measurement increases in importance when children with their maturing physical and psychological potentials are considered. Children's emotions and reflections may be quite different from that of adults. Thus, the effect of asthma on emotions, behaviors or in a broader sense the quality of life should also be measured. For this purpose, asthma quality of life questionnaires was produced (3,6).

Our purpose was to measure the quality of life in children with asthma and to evaluate the association of the Standardized Pediatric Asthma Quality of Life Questionnaire (PAQLQ(S)) with the Childhood Asthma Control Test (c-ACT) and GINA criteria of asthma control. The secondary aim was to determine the association of c-ACT and GINA criteria of asthma control with the PAQLQ(S) domains.

MATERIAL AND METHODS

We planned the study in a cross-sectional design in the Pediatric Allergy and Immunology Clinics of Mersin City and Adana City Research and Training Hospital in Turkey. Mersin and Adana are the cities on the south coast of Turkey, neighbouring the Mediterranean Sea. The population of these cities is about 3,5 million.

Parents of children between the ages of 7-11 who had been followed up at least one year with a diagnosis of asthma according to GINA in the Pediatric Allergy and Immunology Clinics of Mersin City and Adana City Research and Training Hospital were invited to participate in the study. Children whose parents gave informed consent and who fulfilled inclusion criteria enrolled consecutively as they were administered to the Pediatric Allergy and Immunology Clinics in the study period from January to June. Children who were not able to communicate due to language barriers and had chronic diseases other than asthma were excluded from the study. The xxxxxx University Ethical Board approved the study (No:148/2015). Informed consent was obtained from both parents at enrollment.

In order to determine the sample size, the mean of the sum of the quality-of-life scores of those who were controlled according to GINA was found to be 5.5(±2) in the literature. Accordingly, it was predicted that the quality-of-life scores of the uncontrolled subjects would be 25% lower than those of the controlled 4.1(±1.7) and the sample size was calculated accordingly, and it was calculated that there should be 40 patients in each group.

Standardized Pediatric Asthma Quality of Life Questionnaire: PAQLQ was developed for measuring the asthma-related quality of life impairment in children by Juniper et al. in 1996 (7). The activity domain PAQLQ had two generics and three child-specific questions. Because of the three child-specific activities, PAQLQ was not very practical(6). For that reason, PAQLQ(S) and The Mini Asthma Quality of Life Questionnaire-MiniPAQLQ were developed (8) and validated (9). All the activity questions in PAQLQ(S) were generic. The Turkish versions of PAQLQs were validated by Yuksel et al (10).

We preferred to use the self-administered format of PAQLQ(S) in the study (7,8). We used the validated Turkish version of PAQLQ(S) (9). PAQLQ(S) is composed of 23 questions in three domains: Symptoms (10 questions), Activity Limitations (5 questions) and Emotional Function (8 questions). Children recalled their experiences in the last week and responded to each question on a 7-point scale (1= severe impairment, 7= no impairment). The scores were calculated separately for each domain as the arithmetic mean. The total PAQLQ(S) score was calculated as the sum of the scores of the three domains divided by three (6).

Childhood Asthma Control Test: c-ACT is composed of two parts with seven questions in total. The first part‒consisted of four questions which were answered on a scale using a boy's face with emotions to facilitate comprehension by children. The second part consisted of parents answering three questions. The highest score of c-ACT was 27. Children who scored ≤19 points were determined to have uncontrolled asthma, and those who scored >20 were assessed to have controlled asthma (4). A Turkish version of c-ACT was validated by Sekerel (11) et al in 2012.

Demographic data of children (age, sex, atopic sensitizations and the number of emergency visits or hospitalizations in the previous year) were recorded from their medical charts.

Firstly, a clinical nurse gave children the self-administered version of PAQLQ(S). Children were encouraged to complete all of the questions in the PAQLQ(S). Children completed PAQLQ(S) on their own while their parents were waiting in another room for not to distract their answers. After the completion of the questionnaire, the clinical nurse checked whether there were any missing answers. If there were any, she reminded the children to fill out the missing questions. When

children completed PAQLQ(S), she gave them c-ACT and reminded them to fill out the first four questions of c-ACT. After completion of c-ACT by children, parents were asked to answer the remaining three questions of c-ACT according to the symptom frequency of their children in the last four weeks.

After PAQLQ(S) and c-ACT had been completed, children's asthma control was determined according to GINA criteria at the same clinical visit by the same physician.

Statistical Analysis: First of all, the descriptive properties of the variables (mean, median, number and percentage) were found. It was checked whether the numerical variables fit the normal distribution. When comparing the two groups, the student's t-test was used for normally distributed numerical variables, and the Mann-Whitney U test was used for non-normally distributed numerical variables. In cases where more than two variables were compared, ANOVA was used for those with normal distribution, and the Kruskal Wallis test for those who were not normally distributed. Chi-square was used when comparing categorical variables and the Spearman correlation analysis method was used for correlation analysis. The "Statistical Package for Social Sciences" SPSS 25 (IBM Corp., Armonk, NY, USA) program was used to evaluate the results. A p-value <0.05 was considered significant.

RESULT

In total, 120 children participated in the study. The median age (%25-75) was 10.7(2.7) and 62.5% of the children were male. The other demographic and clinical data of the participants and the median scores of each PAQLQ(S) domain were shown in Table 1.

Table 1. Characteristics of children with asthma

Variable	Value
Age, † y	10.7 ±2.7
Sex, male ‡	75 (62.5)
Aeroallergen sensitization, ‡	111 (92.5)
Exacerbations in the previous year, ‡	54 (45)
0	66 (55)
1	32 (26.9)
2	9 (7.5)
3-6	13 (10.8)
Hospitalizations in the previous year, ‡	
0	109 (90.8)
≥1	11 (9.17)
Childhood asthma control test, †	17.9(4.8)
PAQLQ(S) § scores of the study population‡	
Activity limitations	**5.0(1.4)**
Symptoms	**4.6(1.5)**
Emotional Function	**5.2(1.4)**
Total	**4.9(1.3)**

† median (%25-75), ‡, n(%).§ PAQLQ(S):The Standardised Paediatric Asthma Quality of Life Questionnaire

All children filled out a self-administered version of PAQLQ(S) on their own. The median total PAQLQ(S) scores of the study population were 4.90 (1.3). All children had quality-of-life impairments according to PAQLQ(S) scores except four children.

According to GINA criteria of asthma control, 33.3% of children were controlled (n=40), 33.3% of children were partly-controlled (n=40) and 33.3% of them were uncontrolled (n=40). The comparison of patients' data according to GINA control status was presented in Table 2.

Table 2. The comparison of patients' data according to GINA† control status

	Uncontrolled (n=40)	**Partly-controlled (n=40)**	**Controlled (n=40)**	**P**
Age, ‡ years	10.6 (2.9)	11.4(2.8)	10.1 (2.3)	0.029
Sex, male §	25 (62.5)	22 (55)	28 (70)	0.490
Childhood asthma control test's scores‡	13.5 (4.3)	18.4 (2.6)	21.9 (3.1)	<0.001
Childhood asthma control test§				
Uncontrolled	39 (97.5)	25 (62.5)	6 (15)	<0.001
Controlled	1 (2.5)	15 (37.5)	34 (85)	
PAQLQ(S) ‡,¶				
Symptoms	3.5 (1.4)	5.2 (0.9)	5.2 (1.3)	0.363
Activity limitations	4 (1.3)	5.6(1.2)	5.3 (1.2)	0.226
Emotional Function	4.4 (1.5)	5.5(0.9)	5.5 (1.4)	0.603
Total	4 (1.2)	5.4(0.9)	5.3 (1.2)	0.965
Exacerbation in the previous year §	15(71.4)	17(50)	4 (19)	0.001

†GINA: Global initiative for asthma, ‡median (%25-75), §, n (%), ¶ PAQLQ(S): The Standardised Paediatric Asthma Quality of Life Questionnaire

In order to better interpret the difference between the groups, according to the GINA criteria, we included uncontrolled and partially controlled patients as one group, and controlled patients as the other group, and made the comparison between these two groups. When patients with uncontrolled and partly controlled and those with controlled according to the GINA were compared, there were significant differences between the groups (Table 3).

Table 3. The comparison of patients' data according to the revised GINA† control status

	Uncontrolled + Partly-controlled (n=80)	**Controlled (n=40)**	**P**
Age, ‡ years	11 (2.9)	10.1 (2.3)	0.095
Sex, male §	47 (58.8)	28 (70)	0.317
Childhood asthma control test's scores ‡	16 (4.3)	21.9 (3.1)	<0.001
Childhood asthma control test §			
Uncontrolled	64 (80)	6 (15)	<0.001
Controlled	16 (20)	34 (85)	
PAQLQ(S) ‡,¶			
Symptoms	4.3(1.5)	5.2 (1.3)	0.001
Activity limitations	4.8 (1.5)	5.3 (1.2)	0.05
Emotional Function	5 (1.4)	5.5 (1.4)	0.025
Total	4.7 (1.3)	5.3 (1.2)	0.008
Exacerbation in the previous year §	32 (58.2)	4 (19)	0.005

†GINA: Global initiative for asthma, ‡median (%25-75), §, n(%),¶ PAQLQ(S): The Standardised Paediatric Asthma Quality of Life Questionnaire

All of the study population and their parents performed c-ACT. According to c-ACT, 41.7% of children (n=50) had 20-27 points and therefore, were controlled; 58.3% of them (n=70) had <19 points and were addressed to be uncontrolled. The median c-ACT score was 17.9(4.8). The comparison of patients' data according to c-ACT status was presented in Table 4.

Table 4. The comparison of patients' data according to the Childhood asthma control test status

The Childhood asthma control test	**Uncontrolled (n=70)**	**Controlled (n=50)**	**P**
Age, † years	10.7 (2.9)	10.7 (2.5)	0.850
Sex, male ‡	42 (60)	33 (66)	0.633
Childhood asthma control test's scores†	15.1 (4.1)	21.9 (2.3)	<0.001
Global initiative for asthma control status‡			
Uncontrolled	39 (55.7)	1 (2)	<0.001
Partly controlled	25 (35.7)	15 (30)	
Controlled	6 (8.6)	34 (68)	
PAQLQ(S) †,§			
Symptoms	4.1(1.5)	5.3(1.1)	<0,001
Activity limitations	4.6 (1.4)	5.6 (1.2)	<0.001
Emotional Function	4.8(1.5)	5.6 (1.2)	0.004
Total	4.5(1.3)	5.5(1.0)	<0.001
Exacerbation in the previous year ‡	26 (57.8)	10 (32.3)	0,05

†,median (%25-75); ‡, n (%); §, PAQLQ(S): The Standardised Paediatric Asthma Quality of Life Questionnaire

A positive significant correlation was found between the symptom score of PAQLQ(S) and c-ACT (r=0.667; p<0.001). Therefore, it was observed that the c-ACT score increased as the symptom score increased. A positive significant correlation between PAQLQ(S)'s activity score and c-ACT (r=0.494; p<0.001), a positive significant correlation between PAQLQ(S)'s emotional function score and c-ACT (r=0.518; p<0.001) 0.001), a positive significant correlation (r=0.612; p<0.001) was found between the total score of PAQLQ(S) and c-ACT. A positive significant correlation between symptom score and activity score of PAQLQ(S) (r=0.745; p<0.001), a positive significant correlation between symptom score and emotional score (r=0.797; p<0.001), a positive significant correlation between activity score and emotional score (r=0.930; p<0.001).

DISCUSSION

The present study measured the quality of life in children with asthma by using a self-administered version of PAQLQ(S) and showed that loss of asthma control resulted in significant quality-of-life impairments in children. PAQLQ(S) scores were found to be correlated with both c-ACT scores and GINA criteria of asthma control. However, the association of c-ACT with PAQLQ(S) was stronger than that of c-ACT with GINA criteria of asthma control. Additionally, c-ACT was more significantly associated with the symptom domain of PAQLQ(S) than the other domains.

The present study found that quality of life was significantly impaired in children with asthma. Asthma control was obviously associated with quality of life in children. Quality of life impairment was found to be especially related to the symptoms of asthma. In this study, asthma control was measured separately by using two objective methods. Between those objective methods measuring asthma control, c-ACT seemed to be more significantly associated with quality of

life impairments than GINA criteria in children. This finding may be related that actually c-ACT was also related to emotions in addition to the symptoms. Children answer the first part of c-ACT while looking at a boy's face with emotions. Consideration of emotions by this was may also be the reason for the high correlation between c-ACT and PAQLQ(S) (4).

Quality of life measurement in asthma is important for all age groups, but it should be considered, especially in children (2). Because children cannot express all of the symptoms and emotions related to asthma as well as adults. It is a high probability that activity limitations may frustrate children easily. Children cannot establish cause-and-effect relationships in contrast with adults. That may be the reason for the marked difference in PAQLQ(S) scores between the children with controlled and uncontrolled asthma.

It is very important to control the symptoms of asthma. Poor symptom control is a significant risk factor for future exacerbations, emergency department admissions and hospitalizations(1,12,13). In 2004, GINA executive committee recommended that asthma management should be based on symptom control (14). Control-based management defined by GINA has two domains; symptom control and future risk of adverse outcomes. Symptom control is based on the frequency of daytime and nocturnal symptoms, limitation of activities and use of rescue medications(3). Asthma control may also be determined by using composite scores. c-ACT is a simple and user-friendly tool measuring asthma control in children between the ages 4-11 (4). c-ACT also correlates with the GINA classification of symptom control (3).

The present study exemplified that asthma control either defined by GINA criteria or c-ACT is highly associated with quality of life in children with asthma. This finding also showed that loss of asthma control may significantly impair the quality of life of children by causing symptoms, limiting activities and disturbing emotions. It also emphasizes that we can improve the quality of life of children by controlling their asthma. The association of asthma control with quality of life has been examined in both adults (15,16) and children (17). To our knowledge, there is only one study evaluating the relationship of quality of life and quality of life measurement domains with asthma control using both c-ACT and GINA criteria (18).

c-ACT was also more significantly associated with the symptom domain of PAQLQ(S) than the other domains. This is logical because both c-ACT and GINA criteria were related mostly to the symptoms. The addition of either the activity (r=0.74) or emotion (r=0.79) domain to the symptoms increased the correlation. However, both activity and emotion domains further increased the correlation to a high level (r=0.93). furthermore, the total PAQLQ(S) score had a less strong correlation than the duet domains. The feelings related to activity limitation in the PAQLQ(S) score and the symptom control in the c-ACT or GINA are essentially different. Actually, asthma control, symptoms, activity limitations and emotions are also concepts related to each other in asthma.

Although both c-ACT and GINA criteria of asthma control were correlated with PAQLQ(S), these control measures cannot be used instead of quality-of-life instruments. The present study showed that controlled children according to GINA criteria or c-ACT may also have some impairment in quality-of-life scores. In other words, quality of life measurement may cover the other impacts of asthma on life that are not measured by GINA criteria and c-ACT. Quality-of-life questionnaires are too time-consuming to be part of the routine (19). Therefore, both c-ACT and GINA criteria of asthma control may be improved to cover quality of life, at least the part of the quality of life related to activity limitations and emotional functions in addition to measuring asthma control in children.

One of the strengths of the present study is the use of validated tools. The other strength may be the comparison of each domain of PAQLQ(S) with c-ACT and GINA criteria. We suggest subsequent studies with larger sample sizes in older age groups in children.

CONCLUSION

Asthma affected the quality of life in children. PAQLQ(S) scores decreased significantly when asthma was out of control. We found that both c-ACT and GINA criteria were associated with PAQLQ(S). However; PAQLQ(S) was more significantly associated with c-ACT than GINA criteria. Controlling asthma may improve quality of life. It would be useful in clinical practice if c-ACT or GINA criteria can be improved to cover quality of life, at least the part of the quality of life-related to the symptoms or the duet score of activity limitations and emotional functions.

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RESEARCH ARTICLE

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Difficult Encounters Experienced by Family Physicians and the Coping Methods They Employ: A Cross-sectional Study

ABSTRACT

Objective: The purpose of this study was to determine difficult encounters and the practice of medicine among family physicians (FPs).

Method: The research was conducted as a cross-sectional study using a questionnaire between 15 June and 15 July, 2019. The questionnaire included sociodemographic characteristics, difficult situations that may be encountered, and methods of coping with such situations. Three hundred twenty-five FPs took part in the study.

Results: The FPs reported being most frequently troubled by "requests for unnecessary reports" and "patients requesting unindicated tests". FPs described male gender, age 31-40, possession of a moderate income level, being married, and being a civil servant as the patient characteristics most frequently causing difficulties. The mean length of time spent with an ordinary patient was 7.4±0.1 minutes, but this rose to 12.6±0.3 in case of difficult patients. The coping method most frequently employed by FPs in the face of difficult encounters was empathy. Ninety-two percent of the FPs reported experiencing a communication problem with patients at least once a year. Only 22.5% of FPs reported having taken part in training regarding managing difficult situations.

Conclusion: FPs frequently experience difficult encounters. The most frequent of these involve demanding, frequently presenting patients with numerous complaints. The principal reason for FPs experiencing difficult encounters was found to be problems in the health service. Although empathy was the most frequently employed coping method, a lack of training on the subject was also identified.

Keywords: Delivery of Health Care; Difficult Encounter; Family Practice; Physician-Patient Relations; Primary Health Care

Aile Hekimlerinin Karşılaştığı Zor Durumlar ve Başa Çıkma Yöntemleri: Kesitsel Bir Çalışma

ÖZET

Amaç: Bu çalışma ile aile hekimlerinin zor durumlarla karşılaşma durumlarının ve hekimlik pratiklerin saptanması amaçlanmıştır.

Gereç ve Yöntem: Kesitsel tipteki bu çalışma 15 Haziran-15 Temmuz 2019 tarihleri arasında bir anket formu ile yürütülmüştür. Anket formunda sosyodemografik özellikler, karşılaşılabilecek zor durumlar ve zor durumlarla başa çıkabilme yöntemleri yer almaktadır. Çalışmaya toplam 325 aile hekimi katılmıştır.

Bulgular: Aile hekimleri en sık "gereksiz yere rapor talepleri" ve "endikasyon olmadan tektik isteyen hastaların" kendilerini zorladığını belirtmektedir. Aile hekimlerini zorlayan hasta özellikleri erkek olma, 31-40 yaş arası olma, orta gelir düzeyinde olma, evliler ve devlet memurları olarak bildirilmiştir. Sıradan bir hasta görüşmesi ortalama 7,4±0,1 dakika iken, zor hastalarda 12,6±0,3 dakikaya çıktığı bildirilmiştir. Aile hekimleri zor durumlarla karşılaştıklarında en sık başa çıkma yöntemi olarak empati kurmaya çalıştığını belirtti. Aile hekimlerinin %92'si son 1 yılda en az bir kez hastalarla bir iletişim problemi yaşadıklarını belirtmiştir. Zor durum yönetimi ile ilgili sadece %22,5'i bir eğitime katıldığını belirtti.

Sonuç: Aile hekimleri zor durumlar ile çok sık karşılaşmaktadır. Talepkar, sık başvuran, çok sayıda şikâyeti olanlar hastalar en sık karşılaşılan zor durumlardır. Aile hekimleri tarafından zor karşılaşmaların en önde gelen nedeni olarak sağlık sistemi sorunları gösterilmektedir. Empati kurma en sık kullanılan başa çıkma yöntemi olsa da bu konuda eğitim eksikliği olduğu saptanmıştır.

Anahtar Kelimeler: Sağlık Hizmeti Sunumu; Zor Karşılaşma; Aile Hekimliği; Hekim-Hasta İlişkileri; Birinci Basamak Sağlık Hizmeti

INTRODUCTION

Health services are by their nature highly complex and are provided in an environment of uncertainty. Health service users vary considerably in terms of character, needs, and demands. Problems may from time to time be experienced between patients and health personnel during the provision of services. Indeed, these problems may sometimes develop into violence, aggression, and physical assaults against health personnel (1). Turkey is a country where the patient-physician relationship is difficult. Health workers in the country may be subjected to violence or even killed while doing their jobs (2).

Communication between the patient and physician is of great importance in terms of health. This is because appropriate communication is the simplest and most effective means of preventing potential problems between the patient and physician. However, difficult encounters capable of lowering the quality of communication between patient and physician are inevitable in the provision of health services. Almost all physicians experience varying dimensions and levels of difficulty with patients throughout their working lives. However, patients are generally implicated as the source of difficulty between the patient and physician in the literature (3). Health workers employ terms such as "difficult patient", "hateful patient", or "heart sink patient", to refer to such individuals (4, 5).

The term "difficult patient" is used to refer to patients who cause problems in the normal flow of health service provision. The majority of health workers describe difficult patients as "demanding", "aggressive", "obstinate", "self-damaging", or "seeking to manage the health worker" compared to a "normal patient". Patients who do not cooperate with the health worker, "who constantly find fault with the service provided", "who list symptoms that have nothing whatsoever to do with one another", "who demand unnecessary medications", "who distrust the health worker", or who "possess bulging medical files" are also included in the difficult patient category (3). The characteristics of difficult patients may differ. These depend on several factors, such as the patient's age, sex, social class, sociocultural characteristics, psychological state, and disease (4, 6, 7).

Studies have increasingly come to recognize the idea of the" "difficult patient". However, that label entails both practical and emotional implications, and some authors have therefore elected to concentrate instead on encounters and relationships, referring either to "difficult encounters" or "difficult relations" (8).

Researchers investigating interviews with patients described as difficult report that difficulties occurring during these may not only derive from the patient, but also from factors associated with the physician or the health service. Other reported causes of difficult patient-physician interviews include physician-related factors such as long working hours, weak communication skills, and insufficient professional experience, and factors related to the health service such as performance pressure and changes in health financing (9).

It is estimated at 15-30% of family physician (FP) examinations fall into the "difficult" category (10-12). Physicians who encounter large numbers of difficult patients are reported to have higher probabilities of burnout and associated stress or other adverse outcomes than colleagues with fewer such encounters (9, 13). In addition, while some studies report that physicians provide more inadequate health care for difficult patients, others report that difficult encounters are not associated with worse patient care or higher error rates (9, 13).

Treatment is a process based on the patient-physician relationship. Disruption of this process is one of the greatest obstacles to a high quality health service. It is therefore of great importance that that patient-physician relationship be elucidated from all aspects.

The purpose of this study was to determine difficult encounters and the practice of medicine among FPs. There have been a small number of such studies among FPs in Turkey. With this study, we will have the chance to compare the difficult patient encounters of physicians in Turkey with physicians in other countries. The experiences of physicians in different countries will guide us about the causes and solutions of the problem.

MATERIAL AND METHODS

The population of this descriptive research consisted of 413 FPs working in 143 family health centers in 17 districts in the Turkish province of Samsun. With a population of 1.37 million, Samsun is the largest province on the Black Sea coast in the north of Turkey. No sample was selected in the study, and all FPs actively working in Samsun at the time of the study were contacted. The questionnaires were completed at face-to-face interviews with physicians. The study was conducted between 15 June and 15 July, 2019. The aim of the study was forwarded to the FPs prior to the commencement of the study, at which time it was explained that participation was on a voluntary basis, and verbal consent was obtained. Three hundred twenty-five (78.9%) FPs eventually took part. Approval from the Ondokuz Mayıs University clinical research ethical committee and all administrative permissions were obtained before commencement. (IRB No. OMUKAEK 2017/137).

A questionnaire consisting of 20 questions was employed. This was prepared on the basis of a search of the relevant literature (3, 9, 14, 15). Prior to application, the questionnaire was tested on 10 physicians. This self-report form can be completed in approximately 10 minutes. The first part contains questions about FPs' sociodemographic characteristics and working conditions. The second

part lists difficulties that may be encountered in health service provision, and respondents are asked to indicate how frequently they encounter each one. The third part investigates the frequency with which respondents employ coping methods with difficult encounters. Experience of difficult encounters is evaluated from 1, "very rarely" to 5, "very often". It is presented by combining the "Very Rarely/ Rarely" and "Often/Very Often" options. The extent of agreement with propositions is evaluated from 1, "I strongly disagree" to 5, "I strongly agree". The frequency of employment of coping methods was assessed from 1, "I rarely use this" to 5, "I often use this".

The questionnaire data were transferred onto SPSS 22.0 software and analyzed as number and percentage. Paired t-test was used to compare the examination times of physicians for difficult patients and non-difficult patients.

RESULTS

Men represented 64.9% of the FPs in the study, 88.6% of the participants were married, their mean age was 44.6±0.4 years, and their mean time in the profession was 19.7±0.54 years (Table 1). The frequencies of difficult encounters during FPs' professional experience are shown in Table 2. FPs most frequently reported difficulties resulting from "unnecessary requests for reports" and "patients requesting unindicated tests" (Table 2). When asked about the features of patients causing difficulties for them, FPs predominantly responded men (52.6%); individuals aged 31-40 (43.1%), those with a moderate income level, married individuals (59.4%), and civil servants (37.2%). (Table 3)

Table 1. Family physicians' demographic and professional characteristics

	Number	**Percentage**
Sex		
Male	211	64.9
Female	114	35.1
Marital status		
Married	288	88.6
Single/Widowed/Divorced	37	11.4
Place of employment		
Urban center	144	44.3
Outlying district	146	44.9
Rural	35	10.8
	Mean ±SD	**Median (Min-Max)**
Age	44.6±0.4	45(26-64)
Years in the profession	19.7±0.4	20(1-37)

Table 2. The frequencies at which family physicians' experienced difficult encounters

	Percentage		
	Very rarely/ Rarely	Sometimes	Often/Very often
Patients seeking various health reports (for driving licenses, swimming, running, military service, etc.)	5.5	10.8	83.7
Seeking unindicated tests (inappropriate)	11.3	18.2	70.5
Patient relatives making requests on behalf of a patient in the absence of that individuals	13.2	19.7	67.1
Patients making frequent presentations	19.1	25.8	55.1
Patient seeking medications in the absence of any indication (inappropriate)	22.1	27.4	50.5
Patient seeking sick notes for work in the absence of any indication (inappropriate)	27.4	27.4	45.2
Patients with numerous complaints	36.3	27.7	36.0
Patients with insoluble recurrent complaints	31.3	31.4	37.3
Patients seeking to take advantage of the doctor's good nature	48.5	26.2	25.3
Patients with poor treatment compliance	40.4	36.6	23.0
Poorly behaved (over familiar or impolite) patients	57.6	20.6	21.8
Irritable, angry, aggressive patients	55.7	24.9	19.4
Patients expecting a secondary gain	55.0	26.2	18.8
Psychiatric patients	44.4	37.8	17.8
Patients who are never satisfied (dissatisfied)	57.5	25.6	16.9
Manipulative, deceptive patients	72.9	17.2	9.9
Crying patients	85.8	8.3	5.9
Patients with mental problems	83.4	11.4	5.2
Parents unwilling to have their children vaccinated	84.6	10.8	4.6
Cancer patients	76.6	16.9	6.5
Patients exhibiting sexually inappropriate behavior	95.7	2.2	2.1
Terminal patients (in the final stage of life)	85.5	11.1	3.4
Addicts	92.6	5.3	2.1

Table 3. Distributions of the sociodemographic characteristics of difficult patients in the according to family physicians

Difficult Patients Encountered	Number	Percentage
Sex		
Male	171	52.6
Female	154	47.4
Age group *		
0-10	2	0.6
11-20	20	6.1
21-30	44	13.5
31-40	140	43.1
41-50	103	31.7
51-60	52	16.0
61-70	31	9.5
Socioeconomic level *		
Low	128	39.4
Average	186	57.2
High	38	11.7
Marital status		
Married	193	59.4
Single	132	40.9
Occupation *		
Civil servants	121	37.2
Salaried staff-manual workers	57	17.5
Housewives	56	17.2
Commercial, self-employed	41	12.6
Unemployed	27	8.3
Agricultural workers	26	8.0
Retired	23	7.1
Students	16	4.9
No difference between occupations	53	16.3

* More than one answer was given.

FPs were asked an open-ended question about difficult patients' occupational groups. The most frequent reply to that question (29.5%, n=96) was "teachers". Fifty-two percent of FPs stated that patients' negative attitudes toward health workers may derive from their psychological states. The median number of FPs' patients was 60 (min=8; max=110). The mean time spent per patient was 7.4±0.1 min [median (min-max) = 5(2-20)], rising to 12.6±0.3 in [median (min-max) = 10(2-30)] in case of difficult patients. Duration of examination was significantly longer in difficult patients than in non-difficult patients (Paired t=17.95; p<0.001). Only 33.9% of FPs thought that they were able to devote sufficient time to difficult patients.

The extent of agreement among FPs with propositions concerning health workers and the health system as potential causes of interviews with difficult patients is shown in Table 4. FPs described 'creating empathy' as the method they most frequently used for coping with difficult encounters (Table 5). Forty-seven percent of FPs reported experiencing a communication problem with patients at least once a week (Table 6). Only 9.1% of FPs thought that their communication with patients was inadequate. In addition, 66.2% of FPs had received training on the subject of communication, and 22.5% on the subject of difficult patients/encounters (Table 6). Moreover, 26.3% of FPs considered that receipt of training on the subject of education would not contribute to solving their problems, and 23.2% reported that problems experienced with patients affected their private lives.

Table 4. Extents of agreement among family physicians with propositions regarding causes of difficult encounters

Propositions	1	2	3	4	5
My frequency of negative communication with patients decreased as my professional experience increased.	15.6	16.3	22.1	**22.2**	**23.8**
The problems that patients have with other healthcare professionals adversely affect communication between me and the patient.	13.8	21.3	25.9	**20.9**	**18.1**
I began to describe fewer patients as difficult as my professional experience increased.	23.2	15.0	25.0	**20.9**	**15.9**
A negative experience with one patient affects my behavior toward the next patient.	24.0	19.7	21.6	**15.9**	**18.8**
A patient I describe as difficult might not be difficult for another physician.	28.4	20.9	21.9	**11.3**	**17.5**
Problems with physicians cause patients to be labeled as difficult.	27.5	30.3	21.6	**10.6**	**10.0**
The management of the institution I work for do not produce effective solutions to communication problems I have with patients.	11.0	11.9	19.1	**16.3**	**41.7**
Deficiencies among health providers adversely affect my communication with patients.	10.0	13.1	20.0	**19.4**	**37.5**
Problems associated with the health system (payments, health insurance, examination fees, etc.) adversely affect my communication with patients.	21.2	13.8	20.3	**16.6**	**28.1**
High weekly/monthly working hours cause me to describe more patients as difficult.	22.2	21.6	23.1	**17.5**	**15.6**
I describe patients as difficult because my work is not satisfying.	61.9	15.3	15.0	**4.7**	**3.1**

1. Strongly agree, 2. Agree 3. Neither agree nor disagree 4. Agree 5. Strongly agree

Table 5. Methods employed by family physicians to cope with difficult encounters

	1	2	3	4	5
Establishing empathy	4.6	11.1	27.4	20.0	36.9
Learning the patient's method of coping with his clinical problem	12.6	22.8	35.1	19.7	9.8
The direct approach (reducing communication to a minimum)	18.5	26.5	28.6	16.9	9.5
Recommending that the patient consult another physician	23.4	22.2	29.5	16.6	8.3
Specifying a time and subject matter beforehand	31.4	22.8	31.7	9.8	4.3

1. Rarely used 5. Often used

Table 6. Frequencies of communication problems with patients and receipt of training among family physicians

	No.	Percentage
Frequency of communication problems with patients		
Several times a week	82	25.2
Once a week	71	21.8
Once a month	68	20.9
Once in a few months	78	24.0
None in the past year	26	8.0
Receipt of training on the subject of communication	215	66.2
Receipt of training regarding difficult patients/encounters	73	22.5

DISCUSSION

FPs everywhere in the world from time to time find themselves in difficult encounters (9). A study from the USA reported that almost all the FPs interviewed had patients whom they regarded as unpleasant, difficult, and problematic. Having difficult patients appears to be an almost universal experience among doctors (16). These encounters are generally frustrating for the physician. Patients may also be dissatisfied by these encounters due to their needs and expectations not being met and to unresolved medical problems.

In the present study, FPs reported that the difficult encounters they experienced during their professional practice often consisted of "demanding" patients and their relatives requesting inappropriate (non-indicated) reports, examinations, and drugs. Difficult encounters may be attributable to factors associated with the physician, patient, or medical condition, or a combination thereof (9, 17). Numerous factors may be listed under the headings of patient-related factors that may be evaluated as difficult, including "behavioral issues, conditions, and psychiatric diagnoses". "Angry/argumentative/rude" or "demanding/entitled" patients occupy an important place among the behavioral problems (9). A qualitative study from Israel reported that 'patients with a broad range of "behavioral problems" are the most difficult individuals for the majority of FPs interviewed (8). Physicians in another study performed in the USA most frequently described patients with "multiple problems", "demanding behaviors", and "stay sick behaviors" as difficult (16). In other research, 41% of patients identified as difficult were described as "dependent clinger patients", 18% as continually complaining or demanding, and 18% as seeking to direct the course of or refusing treatment (18). In contrast to studies from other countries, "demanding" patients appear to be more prominent in Turkey. We think that labeling a patient as "demanding" cannot simply be reduced to individual patient characteristics or behaviors or to "poor" doctoring. Demanding patients have attracted considerable interest in clinical terms and also in the sociological literature. While healthcare providers seek to characterize demanding patients and minimize detrimental effects on the clinical encounter; sociologists have rather focused on the social contexts resulting in the label "compulsive" (19). Patients making excessive demands on physicians in Turkey can be explained in terms of several factors, including "sociocultural structure", "the social security system" and "red tape". This is because the process that renders a patient demanding is a dynamic one and is associated with several causes. Numerous factors may play a role in this, such as the costs of drugs purchased without a doctor's prescription being met by the Social Security System after a prescription has been obtained, primary health services being free of charge, and reports recommending rest being regarded as "the most valid way of avoiding going to work or school".

FPs most frequently described patients causing difficulties for them as male, aged 31-40, with an average level of income, married, and civil servants. One study reported that 67.1% of patients described as difficult were women, that their mean age was 57.8±15.2 years, that 62% were elementary school graduates, 27% were single and without children, and 35% were retired (18). Another study of practitioner physicians reported that the majority of patients described as difficult were single, aged over 40, and divorced or widowed women (20). According to Stevens (21), academics, the retired, teachers, the unemployed, the self-employed, housewives, and manual workers are all regarded as difficult patients. A study from Turkey performed under hospital conditions reported that 37% of the

patients described as difficult by health personnel were young adults, 38.7% had an average socioeconomic level, 57.1% were married, 56.3% were men, 35.3% were high school graduates, and 36.2% were public sector workers (22). Two different studies reported that that poor and minority group patients feel powerless due to clinical encounters and more compelled to present themselves to physicians in a positive light compared to white, high socioeconomic-level patients (23, 24). Although existing research suggests that there is no association between the patient's sex, age, sociocultural level, or marital status and health providers' perceptions of difficult encounters (12), we think that further research is needed to explore the possible link between the patient's social position and difficult encounters.

FPs in this study were asked an open-ended question concerning the occupational groups with which they experienced difficult encounters. The most frequent response was "teachers". According to Cline and Hayes, 63% of the 90-120 million Americans who regularly use the internet do so to gather information (25). It has been suggested that the internet and other electronic sources of medical information shape patients' understanding of disease. Difficult encounters are embedded within innumerable sociocultural conditions but, as described here, these conditions account for only part of the turmoil between physicians and patients. Difficult encounters are reported to be most common when patients directly or indirectly challenge the physician's judgment or expertise (16). The reason why teachers emerge as the occupational group most frequently reported among difficult encounters in Turkey may derive from teachers making greater use of the internet to obtain health-related information.

FPs reported spending approximately twice as much time on difficult patients than on "normal patients". However, a significant proportion of FPs also thought they were unable to devote sufficient time to difficult patients. One study reported that physicians who described encountering more difficult patients thought that they provided a lower level of care for patients compared to colleagues who reported fewer difficult patients (10). However, the perception of frequent difficult encounters is also reported not to be associated with poorer quality of patient care or higher error rates (13). However, although FPs spend more time during difficult encounters compared to "normal patients", the fact that they regard the time they spend as insufficient should be interpreted as reflecting physicians' desire for quality in health service delivery.

The propositions with which FPs most frequently expressed agreement were "The frequency of negative communication with patients decreases as one's time in the profession increases" and "The management of the employer institution does not produce effective solutions to communication problems with patients". FPs reported that the frequency of difficult encounters as the length of time spent in the profession increased. Similarly to the present research, another study reported that younger health providers provided experienced difficult encounters more frequently (15). Difficult encounters are associated with various factors deriving from the physician, the patient, the condition, or a combination thereof. Every physician brings his own past, personality, and experience to every patient encounter (26). The authors of one study reported that different character traits can either help or hinder physicians in their encounters with difficult patients. Only a small minority of physicians believe that there is nothing in their character that might contribute to difficult encounters or to their assessing a patient as difficult. Physicians cited personal concerns, being a dominant individual, having an overly critical and judgmental personality, the need to be constantly liked by patients, a defensive personality, and being overly polite as characteristics that can make encounters difficult (8). Weak communication skills on the part of the physician, situational stress factors, and prejudices concerning specific health conditions have also been described as causes of difficult encounters (9). Additionally, encounters are much more difficult when physicians bring their own family lives, social lives, economic problems, and anxieties over problems in the health service to the interview. In the light of the negativities in the health system in Turkey, the economic problems experienced by health workers and the violence inflicted on them, it may be concluded that difficult encounters experienced by physicians are not solely due to difficult patients.

FPs most frequently reported 'establishing empathy' as their main coping method in the context of difficult encounters. A study of FPs from Israel also reported also reported establishing empathy as the most widespread and apparently effective means of coping (8). It is encouraging to see that physicians in Turkey do not reject difficult patients, but that, on the contrary, they seek ways of improving difficult medical encounters. The majority of solutions lie in the field of proper communication and improving patient-doctor relationships. FPs recognize the importance of empathy listening without judging, patience, and tolerance. Such methods are frequently recommended in medical interviews in general, and particularly in coping with difficult patients (9, 27, 28). However, the responses of family physicians may be conditioned by the bias of social desirability. This response about using empathy in difficult encounters may reflect the "desire" to do so rather than the fact that they actually do. It is unlikely that a FP, faced with emotional distress caused by a difficult encounter with a patient, will be able to connect and understand the patient's

feelings and also communicate it appropriately. Feelings of helplessness, anger, or being overwhelmed by FPs would make it difficult for them to face the situation with empathy. In fact, when difficult encounters are studied through audio or video recordings, empathy is conspicuous by its absence, as in the case of patients with medically unexplained symptoms (29).

FPs reported experiencing communication problems with patients at least once a week, although the great majority regarded themselves as proficient on the subject of communicating with patients. More than half of FPs had received training on the subject of communication, but the proportion who had received training regarding difficult patients or encounters was low. Communicating with the patient is highly important in achieving the desired therapeutic results. Studies report that the patient's satisfaction with the service provided is largely dependent on the behavior of the healthcare worker. In addition, a patient leaving happy with the interview that has taken place also ensure the satisfaction of the health workers are reduces occupational stresses (30-32). Some authors believe that the patient is usually the source of physician-patient conflict (33). However, physicians themselves can also be responsible for difficult encounters (14). Even if patients do cause communication problems, physicians need to possess the professional communication skills to allow them to cope with difficult encounters (34). Studies involving physicians report that education is effective in the management of encounters with difficult patients (35, 36). However, the majority of medical education programs do not involve structured or specific approaches to developing communication skills (37). We therefore think that FPs in particular should receive regular training on the subject of communication and coping with difficult encounters.

CONCLUSION

FPs frequently experience difficult encounters. These encounters most commonly involve patients who are demanding, who make frequent presentations, and those with numerous complaints. FPs regard problems in the health service as the leading reason for difficult encounters. Establishing empathy is the most frequently employed coping method. FPs should receive regular training on communication and coping with difficult encounters.

Limitations: There have been no previous such studies among FPs in Turkey. FPs participation in the study is high. However, the study was conducted in a single province and only with physicians. Patients' opinions on this issue were not taken. What is really needed to advance this field of research is the performance of more cohort studies, the use of objective measures of the difficult encounter (audio or video-recordings) and the simultaneous study of the opinion of the patient and FP in the same difficult encounter.

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CASE REPORT

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Emergency Surgery due to Giant Diaphragm Hernia Causing Acute Abdominal and Dyspnea: A Case Report

ABSTRACT

A 78-year-old female patient was admitted to the emergency department with severe abdominal pain and respiratory distress. In the Computer Tomography (CT) examination of the patient; It was observed that there was a hernia in the left leaf of the diaphragm and the stomach, spleen and intestines were protruding in the left pleural space up to the left clavicle. In the patient's anamnesis; there was breathing difficulty that has been going on for many years and has increased in recent days and the general condition disorder that has developed recently. A giant left diaphragmatic hernia was observed in our patient, who was urgently scheduled for diagnostic dslaparotomy. The spleen, stomach and intestines in the left pleural cavity were taken into the abdomen and the diaphragm defect was repaired.

Keywords: Atraumatic Diaphragmatic Hernia, Acute Abdominal, Dyspnea

Akut Batın ve Dispneye Neden Olan Dev Diyafram Fıtığına Bağlı Acil Cerrahi: Olgu Sunumu

ÖZET

78 yaşında kadın hasta şiddetli karın ağrısı ve solunum sıkıntısı ile acil servise başvurdu. Hastanın Bilgisayarlı Tomografi (BT) incelemesinde; Diyaframın sol yaprağında fıtık olduğu, sol plevral boşlukta sol klavikulaya kadar mide, dalak ve bağırsakların çıkıntı yaptığı görüldü. Hastanın anamnezinde; uzun yıllardır devam eden ve son günlerde artan nefes alma güçlüğü ve son zamanlarda gelişen genel durum bozukluğu vardı. Acilen tanısal laparotomi planlanan hastamızda dev bir sol diyafragma hernisi görüldü. Sol plevral boşluktaki dalak, mide ve bağırsaklar karın içine alınarak diyafram defekti onarıldı.

Anahtar Kelimeler: Atravmatik Diyafram Fıtığı, Akut Abdominal, Dispne

INTRODUCTION

Most congenital diaphragmatic hernias develop from the left posterolateral foramen and are defined as Bochdalek hernias. Morgagni hernia is a congenital defect that occurs due to the failure of the sternal and costal elements of the diaphragmatic cruses from the Larrey space and has been reported as 5% in large series(1). Clinical presentation ranges from asymptomatic cases to serious respiratory or gastrointestinal symptoms and sometimes haemodynamic instability (2). The most common contents of the hernia sac include the omentum, followed by the colon, small bowel, stomach, spleen and portions of the liver (3). The present case is a rare case of an adult with Diaphragm Hernia presenting with abdominal pain, dyspnea and synchronous prolapse of the spleen, stomach, small intestine and colon.

CASE REPORT

A 78-year-old male patient was referred to our hospital from a secondary healthcare facility with a pre-diagnosis of strangulated diaphragmatic hernia. In the anamnesis of the patient; it was learned that they applied to the hospital because of the complaints of shortness of breath for fifteen days, increasing their complaints with each passing day and having a general deterioration. There was no history of trauma. Her medical history was unremarkable except for recurrent asthma attacks and heart failure for many years.

Her physical examination was conscious-open and cooperative-orientated. Vital findings were Tension Arterial: 110/75 mmHg, Fever: 37.0 oC, Pulse: 103/min-rhythmic, Respiratory Rate: 25/min-regular, Oxygen Saturation: 91%. In the examination of the abdomen; especially prevalent mainly in the upper quadrant taken tenderness.

When the Computer Tomography (CT) taken in a secondary health institution was examined, It was seen that the spleen and intestines were in the left hemithorax. (Figure 1) Intestinal air is seen in the left hemithorax in the preop chest x-ray taken in our hospital. (Figure 2).

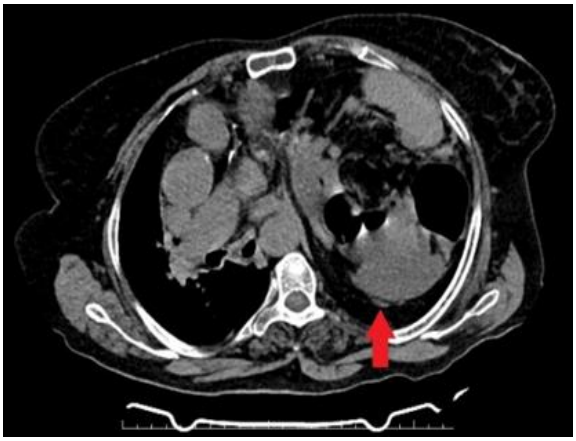

Figure 1. Spleen and intestines seen in the left hemithorax.

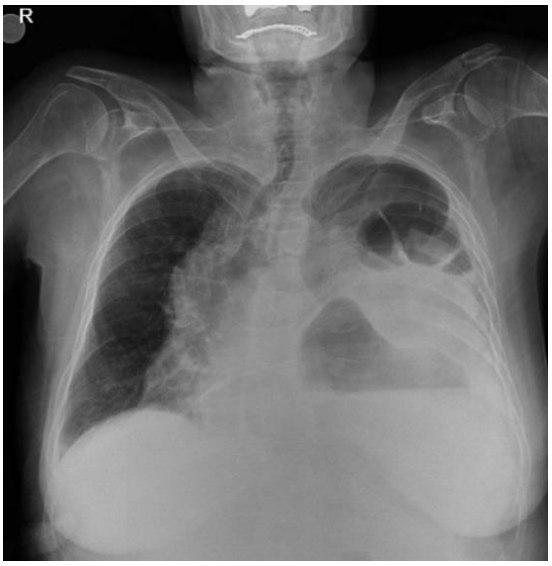

Figure 2. Intestinal air is seen in the left hemithorax.

In our patient's laboratory examination, a slight neutrophil increase (7.8 10^3/uL) and CRP increase (3.3 mg/dL) were observed in the hemogram. Apart from these, we look at the biochemical and hematological parameters were normal.

After that, the patient was operated and diagnostic laparotomy was performed under general anesthesia. In the exploration, it was seen that there was a large defect in the left diaphragm and the intestines and spleen penetrated from this defect. Intestines and spleen were seen in the left hemithorax up to the left clavicle. After the spleen was withdrawn into the abdomen, bleeding was observed in its capsule, and the decision to splenectomy was made because the bleeding did not stop. The intestines were pulled into the abdomen after the splenectomy was completed. Afterwards, a 10x15cm prolene mesh was sutured to the diaphragm one by one using 2-0 prolene and the defect was closed.

In the chest x-ray taken on the postoperative first day, it was seen that the left lung was ventilated completely. (Figure 3)

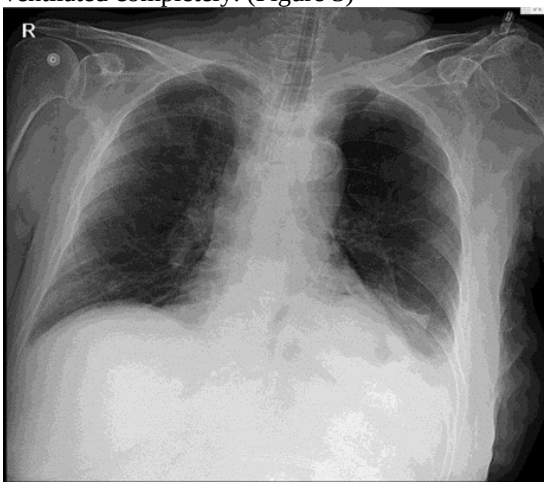

Figure 3. Chest x-ray taken on postop 1st day.

DISCUSSION

Morgagni hernia in adults is more common in women. In a study of 298 patients, 63% of the patients were found to be women. Symptoms were detected with hernia in 72% of the patients. It was observed that male patients complained of symptoms related to hernia more and earlier than female patients (4).

Diaphragm Hernia usually constitute only the omentum in infants and children, but with time, the defect enlarges until the abdominal organs herniate through. Pregnancy, trauma, obesity, chronic constipation, and chronic cough are common predisposing conditions contributing to the development of Diaphragm Hernia. Symptomatic patients frequently complain of abdominal or chest pain and respiratory distress (3). In our patient, the diaphragmatic hernia expanded and turned into a giant, possibly due to increased intra-abdominal pressure due to asthma attacks. As a result, the patient appeared with severe dyspnea and abdominal pain symptoms.

Morgagni hernia can be repaired with thoracic and abdominal access. Abdominal approach is preferred more than thoracic approach. In addition to open surgery in the transabdominal approach, laparoscopic surgeries have been successfully performed for many years (4). Although we have laparoscopic experience in our clinic on behalf of diaphragmatic hernias, the operation that started laparoscopically continued as open surgery because the patient had a giant hernia and the spleen and intestines were not reduced into the abdomen.

Although there are limited studies in the literature on mesh or meshless repair of diaphragm defect, the general opinion is that recurrence is less in mesh repair (5). While the literature review suggested repairing the defect using a mesh, some preferred suture repair. The choice of repair depends on the size of the defect, as larger defects will usually not be able to be repaired by suture (6). In our case, the defect was repaired with prolene mesh, considering the presence of a giant diaphragmatic hernia and the absence of recurrence.

RESULT

When symptoms suggestive of gastrointestinal obstruction are encountered with respiratory distress, diaphragmatic hernia should be included in the differential diagnosis. In such cases, a chest X-ray or a CT to be seen more clearly may be ordered to support the diagnosis. As in our case, it should not be delayed in the emergency operation of a giant diaphragmatic hernia that gives symptoms. But more importantly, it should be kept in mind that the operation of asymptomatic diaphragmatic hernias detected incidentally will prevent possible complications. In addition, we think that repairing the defect with mesh has an important role in terms of recurrence.

Written informed consent form was obtained from the patient. The authors declare that there is no conflict of interest.

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