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EDITORIAL

EDİTÖRYAL

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Scientificity and H-Index

Bilimsellik ve H-Endeksi

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ABSTRACT

Scientificity is a comprehensive expression that identifies the scientific contribution of an academician or a scientist to the literature. It is established on the basis of certain determinants, such as the h-index, which is one of the indicators used to evaluate the degree of scientificity in a contribution. This editorial explains the h-index and its necessity.

Keywords: Scientificity, indicator, h-index

ÖZ

Bilimsellik, bir akademisyenin veya bir bilim adamının literatüre bilimsel katkısını tanımlayan kapsamlı bir ifadedir. Bilimselliği saptamak için kabul edilen bazı belirleyiciler vardır. h-endeksi, katkıda bulunanların bilimsel seviyesinin tespiti için hali hazırda kullanılan göstergelerden biridir. Bu yazıda h-indeksi ve gerekliliğini açıklamaya odaklandık.

Anahtar Kelimeler; Bilimsellik, belirteç, h-indeks

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The literature expands day by day owing to contributions and recent advancements from researchers. However, the publication of a paper does not mean that the material is a scientific contribution [1]. This issue prompted the identification of factors that determine the scientificity of a scientist's contribution or report [1]. Some of these determinants are the total number of papers published by scholars, the total citations or citation rates per article and qualified publication counts. The problem with these indicators is that they are not internationally standardised parameters, driving the development of the h-index to acquire a standardised value that combines all the aforementioned parameters in an equation [1–3].

Jorge E. Hirsch developed the h-index in 2005 to

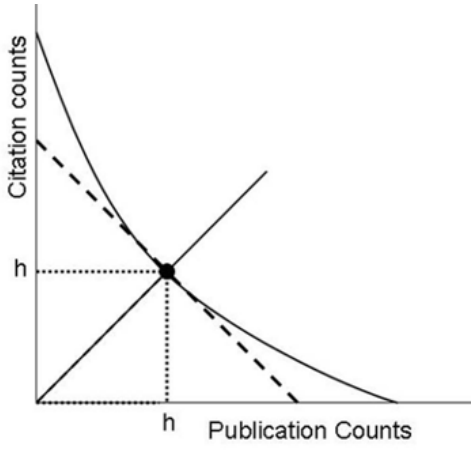
resolve the insufficiencies of previous indices [4]. This index is intended for use in the evaluation of both publication activity and citation rate per publication. It roughly indicates that an author has Np number of articles with at least h citations. For instance, a scientist with an h-index of 10 has had 10 articles published, which has been cited in at least 10 separate instances, or a scientist with an h-index of 5 has had published at least five manuscripts that have been accorded over five citations. For each point of increase in the h-index, the first five articles would require one more citation each, and one article other than the five needs more citations than that achieved by the first five articles [2].

The h-index initially elicited attention from other scientists, who positively evaluated it, commending its efficacy. Nevertheless, other reports asserted that a single index cannot reflect the full scientificity of a scientist's contributions [2,5]. Certain scholars also criticised the necessity of time in the use of the h-index, arguing that the importance of new research cannot be evaluated using this parameter. These shortcomings motivated improvement to the h-index, albeit it remains the most widely accepted and applied indicator of scientificity [5].

Hirsch explained the h-index with a diagram (Figure 1) and an equation [2,3], but recent applications reflected that it can be calculated automatically by the electronic scanning motors in the Web of Science (WoS; administered by Clarivate Analytics) database; other databases, such as Chemical Abstracts Services (Columbus, OH, USA), Google Scholar and Scopus, can automatically calculate this parameter according to the listing of articles and entire citations in the systems [6]. Each one of these databases produces a different h-index score for the same academic institution or publications because of variations in the number of citations.

The h-index can be used to identify a researcher's scientific achievement, but it can also be employed to ascertain the level of scientificity in groups or organisations. Hereby, universities, publishers, institutes and countries started using the index to determine and monitor the scientificity of their facilities and the endeavours that they pursue in their current positions. Owing to the advanced usage of the h-index, it became an approved indicator of impact factor [6]. Recently, reviewers affiliated with organisations with high impact factors have been selected to evaluate manuscripts submitted to our journal, and editors monitor the scientific achievements evident in articles published in the Journal of Acta Medica Alanya on the grounds of the h-index.

Figure 1. Calculating the h-index (source: Hirsch [4])



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RESEARCH ARTICLE

ARAŞTIRMA

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Comparison of efficacy of platelet-rich plasma and extracorporeal shock-wave therapy on pain and functional capacity in patients with Plantar Fasciitis

Plantar Fasiit Hastalarında Trombositten Zengin Plazma ve Ekstrakorporeal Şok Dalga Tedavisinin Ağrı ve Fonksiyonel Kapasite Üzerine Etkisinin Karşılaştırılması

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ABSTRACT

Aim: Plantar Fasciitis (PF) is one of the most common causes of heel pain. There are various conservative methods for the treatment of PF. Nearly 10% of the patients are refractory to the conservative methods and thus undergo surgical procedures. This study aimed to compare the efficacy of platelet-rich plasma (PRP) injection with that of extracorporeal shock-wave therapy (ESWT) in PF patients.

Patients and Methods: The study included 58 patients (40 females), who had complaints for at least 3 months and were refractory to the non-steroidal anti-inflammatory drugs and stretching exercises. The patients who underwent PRP injection (n=29) and ESWT (n=29) were evaluated regarding pain severity by Visual Analogue Scale (VAS) and functionality by Foot Function Index (FFI) both before and at the 3rd month after treatment.

Results: In both groups, a significant improvement was observed in the VAS and FFI scores at the 3rd month after the treatment as compared with the pretreatment scores. The improvement in the VAS and FFI scores was significantly greater in the PRP group than in the ESWT group ($P < 0.05$).

Conclusions: PRP can be a more effective method than ESWT in improving pain and functional scores in PF patients. However, further studies are needed on this issue.

Keywords: Platelet-rich plasma, Plantar Fasciitis, extracorporeal shock-wave therapy, pain, injection, therapy, foot joints, complementary medicine

ÖZ

Amaç: Plantar fasiit (PF), topuk ağrısının en sık görülen nedenlerinden biridir. PF tedavisi için çeşitli konservatif yöntemler vardır. Hastaların yaklaşık% 10'u konservatif yöntemlere dirençlidir ve bu nedenle cerrahi işlemlere tabi tutulur. Bu çalışmada PF hastalarında trombositten zengin plazma (PRP) enjeksiyonunun ekstrakorporeal şok dalgası tedavisinin (ESWT) etkinliği ile karşılaştırılması amaçlandı.

Hastalar ve Yöntemler: Çalışmaya en az 3 aydır şikayeti olan ve steroid olmayan antienflamatuar ilaçlara ve germe egzersizlerine direnç gösteren 58 hasta (40 kadın) alındı. PRP enjeksiyonu (n = 29) veya ESWT (n = 29) uygulanan hastalar, tedavi öncesi ve tedaviden sonraki 3. ayda, Ağrı şiddeti, Görsel Analog Skala (VAS) ve İşlevsellik ise, Ayak Fonksiyon İndeksi (FFI) ile değerlendirildi.

Bulgular: Her iki grupta da, tedavi sonrası 3. ayda VAS ve FFI skorlarında tedavi öncesi skorlara göre anlamlı bir iyileşme gözlendi. PRP grubunda VAS ve FFI skorlarındaki iyileşme ESWT grubuna göre anlamlı olarak daha yüksekti ($P < 0.05$).

Sonuç: PRP, PF hastalarında ağrı ve fonksiyonel skorları iyileştirmede ESWT'den daha etkili bir yöntem olabilir. Ancak, bu konuda daha fazla çalışmaya ihtiyaç var.

Anahtar kelimeler: Trombosit bakımından zengin plazma, Plantar fasiit, ekstrakorporeal şok dalgası tedavisi, ağrı, enjeksiyon, tedavi, ayak eklemleri, tamamlayıcı tıp

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INTRODUCTION

Plantar Fasciitis (PF) is known as the most common cause of heel pain in adults. PF accounts for nearly 80% of the heel pain in people. Moreover, it has been found that one of each ten people experiences heel pain at some point during their lives [1].

The most widely accepted opinion regarding the etiology of PF is that PF is not an inflammation but a degenerative process due to myxoid degeneration, micro lacerations, collagen necrosis, and angiofibroblastic hyperplasia resulting from repetitive microtraumas, particularly at the calcaneal attachment point of the Plantar fascia [2]. The pain typically occurs in the morning and/or with the first step after sitting for a long time and causes difficulties in performing the activities of daily living [3]. The diagnosis is usually established based on the clinical history and the signs of local tenderness.

PF is frequently a self-limiting disorder. Complaints disappear in 10 months in 80-90% of the cases. Nevertheless, the process can be problematic for both patients and physicians. Depending on the natural course of the disease, the choice of treatment is usually non-surgical therapeutic options [4]. Conservative methods, including non-steroidal anti-inflammatory drugs (NSAIDs), heel cushions or orthoses, physical therapy, stretching exercises, corticosteroid injection, and extracorporeal shock-wave therapy (ESWT), form the basis of PF treatment and provide significant relief in nearly 80% of the patients [5].

ESWT has brought a new dimension to the treatment of PF. In the year 2000, the US Food and Drug Administration (FDA) approved the use of a ESWT device, which is an electro-hydraulic device, in the treatment of chronic PF [6]. Shock-wave modalities can create rapidly rising acoustic waves by their high peak-pressure amplitudes and most of the energy flow is concentrated on a small focus [3]. Pain, redness, edema and ecchymosis are quite rarely reported during ESWT procedure and are not permanent. The exact mechanism of action of ESWT remains unclear. However, it is thought to act by stimulating the repair process of the body and by enhancing healing ability in the relevant region.

In the treatment of PF, ESWT shows its efficacy over the biological mechanisms by destroying unmyelinated sensory nerve fibers and thereby initiating the neovascularization process in the degenerative tissues. Previous studies have reported that ESWT stimulates secretion of local growth factors and accelerates intrinsic wound healing processes such as selection of suitable stem cells [5].

Today, another non-surgical therapeutic option for PF is the platelet-rich plasma (PRP) injection. PRP is a treatment modality that stimulates natural healing steps via the growth factors in platelets. PRP applied to a wound site accelerates healing process, provides support for cellular binding, reduces pain, and has anti-inflammatory and anti-bacterial effects [7]. In the literature, there are studies investigating the use of PRP in the treatments of PF and chronic tendinopathy. In addition to the ready-to-use kits, PRP can be obtained also manually from the peripheral blood.

Nevertheless, many questions about the PRP procedure such as ideal volume, frequency of application, and platelet activation have remained unanswered [8]. Thus, the present study aimed to investigate the effects of ESWT and PRP injections which have been frequently used in recent years for the treatment of PF patients on pain and physical functioning.

The present study included patients who were admitted to the Physical Medicine and Rehabilitation Outpatient Clinic between January 2018 and July 2018, diagnosed and followed-up with PF, and refractory to the first-line conservative treatment consisting of NSAIDs and stretching exercises performed for at least three months.

The patients were randomly (sealed envelope) divided into two groups to undergo PRP injection or ESWT. The diagnosis of PF was established based on clinical examination and plain radiographies of the patients were reviewed to distinguish other pathologies of the heel.

Exclusion criteria were as follows: presence of systemic diseases, pregnancy, presence of active tumor or hematological malignant disease, presence of infection, history of anticoagulant use, receiving NSAIDs within the past 5 days,

a hemoglobin level of <11 g/dL, a platelet count of <150,000/mm3, history of previous steroid injection into the heel region, presence of a previous calcaneus fracture, or history of previous surgery of the heel region.

The study was approved by the Institutional Review Board of Medicana International Istanbul Hospital (IRB 01-2018-005). All patients were informed about the study procedures, their consents were obtained and the patients' personal rights were protected over the study period by following the Helsinki Declaration.

Platelet-rich plasma injection and extracorporeal shock-wave therapy procedures:

Preparation and application of PRP were performed under the same conditions in all patients. A total of 10 mL of peripheral blood was drawn from the antecubital vein into the tubes containing 3.2% sodium citrate. The blood samples were centrifuged at 3200 rpm for 10 minutes at room temperature using an Eppendorf® centrifuge 5702 device (Hamburg, Germany).

The obtained 2 mL of PRP was administered under sterile conditions into the most sensitive point determined by palpation in the medial region of the foot. After PRP administration, the patients were placed in the supine position for 20 minutes. Each patient was informed that the pain would probably increase for the next three days. Ice application was recommended on the painful region for 10 minutes 3 times a day. In addition, the patients were asked not to receive NSAIDs for one week as they are likely to interfere with platelet activity. Additional treatments, such as orthoses (for example heel cushion) were not performed until the end of the study. PRP injection was performed 3 times with 1-week intervals.

In the ESWT group, ESWT was performed on the region where the pain was the most intense using the BTL-6000 SWT device (BTL Industries Ltd., Hertfordshire, UK) with a frequency of 15 Hz, at a pressure of 2 bars, and with 2 500 pulse/session. This procedure was performed once a week for three weeks (three sessions). The patients in the ESWT group were also recommended to apply ice in the presence of pain. Additional treatments, such as orthoses and heel cushions, were not performed until the end of the study.

All patients in both groups were provided with a six-week exercise program. This program consisted of stretching exercises for the gastrocnemius and soleus muscles and the Plantar fascia. The exercises were performed under the supervision of the same clinician as 15 repetitions each for 10 seconds and twice a day. The patients were evaluated both before and 3 months after the treatment using the Visual Analog Scale (VAS) for pain and the Foot Function Index (FFI) for functional status.

Pain Assessment:

For pain assessment, the pain felt during the first few steps in the morning was evaluated using the VAS. A 10 cm non-segmented line was used to assess the pain severity. It was explained to the patient that 0 corresponds to no pain and 10 corresponds to unbearable pain. The patients were asked to mark the point on the non-segmented line indicating the severest pain felt during the first few steps in the morning and the values were recorded in the patient's follow-up form.

Functional Assessment:

Functional assessment was performed using the FFI which is used for foot problems affecting foot functions. The FFI is a questionnaire consisting of 23 items divided in to 3 subcategories (5 items in the activity limitations subcategory, 9 items in the pain severity subcategory and 9 items in the disability subcategory). Each item was evaluated by marking on a 10-cm horizontal line according to the VAS and was rated between 0 and 100. The arithmetical mean of all scores was calculated: higher average scores indicated higher pain, disability and activity limitation [9]. The Turkish translation and adaptation of the FFI was performed by Yalıman et al [10].

For both VAS and FFI, values were recorded at the beginning of treatment and 3 months after the end of treatment

Statistical Analysis:

Data analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) and the normality of the

data was tested using the Kolmogorov-Smirnov test. Descriptive statistics were expressed as mean, standard deviation, median, minimum and maximum, and number and percentages, where appropriate. The independent samples t-test and the Mann-Whitney U test were used to compare quantitative data. The Wilcoxon test was used for the analysis of repeated measurements and the chi-square test was used to compare qualitative data. A P value of <0.05 was considered statistically significant.

RESULTS

The present study included 58 patients with PF (40 females and 18 males) who were divided into two groups as those undergoing PRP injection (PRP group; n=29) and those undergoing ESWT (ESWT group; n=29). The mean age of the PRP group (20 [69.0%] females and 9 [31.0%] males) was 47.4±11.1 years (median, 48.0 years) and the mean age of the ESWT group (20 [69.0%] females and 9 [31.0%] males) was 49.9±8.2 years (median, 49.0 years). The study groups were similar in terms of age (P = 0.337) and sex (P = 1.000) distribution. None of the patients developed local or systemic complications during or after the procedures (Table 1).

The VAS and FFI scores of the PRP and ESWT groups, before and after treatments as well as the change in the scores from pre- to post-treatment, are presented in Table 1. No significant difference was determined between the groups in terms of pre-treatment VAS scores (P >0.05).

The mean VAS score was observed to be significantly decreased from pre to post-treatment, both in the PRP (P <0.05) and ESWT groups (P <0.05). The mean post-treatment VAS score was significantly lower in the PRP group than in the ESWT group (P <0.05). The decrease in the mean VAS score from pre to post-treatment was significantly higher in the PRP group than in the ESWT group (P <0.05).

The PRP and ESWT groups did not differ in terms of pre-treatment FFI scores (P >0.05). The mean FFI score was observed to be significantly decreased from pre to post-treatment both in the PRP (P <0.05) and ESWT (P <0.05) groups. The mean post-treatment FFI score was significantly lower in the PRP group than in the ESWT group (P <0.05). The decrease in the mean FFI score from pre to post-treatment was significantly higher in the PRP group than in the ESWT group (P <0.05) (Table 1).

Table 1. Visual Analog Scale scores and Foot Function Index scores of the study groups

		PRP Group		ESWT Group		P
		Mean±s-d/n-%	Median	Mean±s-d/n-%	Medi-an	
Age		47,4 ±11,1	48	49,9 ±8,2	49	0,337t
Gender	Fe-male	20/ 69,0%		20/ 69,0%		
	Male	9/ 31,0%		9/ 31,0%		
Visual Analog Scale (VAS)						
Pre-Treatment		6,6±1,3	7,0	6,4±1,4	7,0	0,594m
Post-Treatment (3 Month)		2,0±1,6	2,0	3,5±1,5	4,0	0,001m
Post-Treatment Change		4,7±2,1	5,0	2,9±1,4	3,0	0,001m
Difference Intra Group p		0,000	w	0,000	w	
Foot Function Index (FFI)						
Pre-Treatment		69,0±12,9	70,8	66,9±16,1	69,8	0,675m
Post-Treatment (3 Month)		21,7±16,8	21,6	36,3±17,5	40,0	0,006m
Post-Treatment Change		47,3±20,4	50,0	30,6±17,8	32,0	0,005m
Difference Intra Group p		0,000	w	0,000	w	

[t] t test / [m] Mann-Whitney u test / [X²] Chi-square test / [w] Wilcoxon test

PRP, platelet-rich plasma; ESWT, extracorporeal shock-wave therapy; SD, standard deviation; VAS, Visual Analog Scale; FFI, Foot Function Index.

DISCUSSION

Plantar Fasciitis, which is the most common cause of heel pain in adults, appears in one of each 10 people at some point during their lives and the sooner the treatment is started the faster it improves. PF has been reported to be more prevalent in females, overweight individuals, and young male athletes [5]. In the present study, the percentage of female patients (69%) was also higher.

In the literature, it has been reported that PF occurs at the age of 25-64 years and is most frequently

seen at the age of 40-60 years [1]. In the present study, the mean age was 48.7±9.8 years and was in line with those reported in the literature.

Treatment of PF consists of two groups of approaches as conservative and surgical treatments [11]. Nevertheless, there is a remarkable consensus on the fact that non-surgical techniques would be sufficient in 70-90% of the patients with heel pain [5,11]. The first-line treatment protocol includes the following, in order of use: resting and activity modification, cold compress, stretching techniques, NSAIDs, heel cushions and insoles, and weight loss [2]. In case sufficient improvement could not be achieved in 6-8 weeks, a second-line treatment, including physical therapy implementations (strengthening exercises, iontophoresis, deep myofascial massage), injection therapies (steroids, dextrose, botulinum toxin, PRP), ESWT, dry needling and night splints, is used after the diagnosis is confirmed using imaging methods [12]. Plantar fasciotomy is recommended for persistent PF, which is refractory to conservative therapies and complaints of which last for longer than 6 months [1]. There are numerous non-surgical treatment options with different success rates in the treatment of PF, yet there is currently no consensus on the ideal choice of treatment for PF.

In recent years, PRP and ESWT have been increasingly used for the treatment of the pathologies of foot and ankle. The present study was designed to compare the efficacy of PRP injections with that of ESWT in the treatment of chronic PF. Accordingly, 3-dose PRP injections in the treatment of PF was determined to be more effective than 3-sessions of ESWT in terms of pain and functional outcomes.

The average platelet concentration of whole blood is 200,000 per µl (normal range 150,000–350,000 per µl). Platelets are small anucleated cytoplasmic fragments of megakaryocytes that are commonly thought of as the responsible agents for hemostasis. Although the platelet is central to the coagulation cascade, it is also essential to tissue healing. The first step of the healing process is clot formation and platelet activation. Many growth and differentiation factors are released from the α-granules, which are the storage units found in platelets. 95% of the existing factors are released within 10 min of clot formation, whereas the rest of the growth factors are released as they are formed over several days. In vivo and in vitro researches also suggest that PRP induces over expression of additional endogenous growth factors, beyond what is contained within the platelet concentrate [13,14]. PRP activates the circulating regenerative cells by stimulating various types of cells in the tissues [15]. There are more than 30 bioactive proteins in the alpha granules of the platelets. Growth factors in PRP such as platelet-related growth factors, transforming growth factor, vascular endothelial growth factor and insulin-like growth factor, as well as proteins in PRP such as fibrin, fibronectin, vitronectin, and thrombospondin, play a role in many steps of tissue healing [10]. Growth factors provide soft tissue healing and bone regeneration by activating some cells that play a role in tissue healing. With the effects of growth factors which blood includes, PRP stimulates the local stem cells and activates the regenerative cells in the bone marrow through blood circulation. In addition, regarding tendon healing, PRP enhances tenocyte proliferation in the healing site by providing revascularization via the growth factors it includes and plays an effective role in enhancing collagen expression in the tenocytes [16].

PRP can be obtained either as ready-to-use kits or manually. In a vitro study, it was stated that a thrombocyte concentration 2.5 times higher than the basal thrombocyte count would be the most effective [17]. A prepared PRP sample is activated with the addition of bovine or human thrombin or calcium chloride. Growth factors and cytokines are released from the activated PRP with the formation of thrombocyte gel. In some previous studies, PRP has been used without activating. There is no consensus on the ideal volume, frequency of application or thrombocyte activation in PRP procedure [8]. Martinelli et al. applied PRP treatment to 14 patients with PF at 1-week interval, 3 sessions in total and found improvement in pain and functional scores in all patients after 12 weeks [18]. In the present study PRP injection was performed 3 times with 1-week intervals, prepared using a ready-to-use kit (S&M PRP kits, STR Biotechnologies Co. Ltd., Çorum, Turkey). The analysis of the prepared PRP

sample using this kit revealed a 5-times higher platelet concentration than that in the peripheral blood and calcium chloride was not added into this preparation for activation.

Extracorporeal shock-wave therapy has been used for the last decade in the treatment of chronic PF. It is a debatable method of treatment, despite the presence of numerous studies supporting its clinical efficacy, as neither the underlying mechanism of therapeutic efficacy nor the most appropriate treatment protocol has been completely identified yet [19]. Extracorporeal shock wave therapy (ESWT) is a noninvasive procedure used in rehabilitation therapy that is recently being applied in the treatment of tendinopathies and Plantar Fasciitis as well [20]. In ESWT, shock waves are generated by means of electrohydraulic, piezoelectric and electromagnetic methods. There are some possible mechanisms mentioned for the efficacy of shock wave therapy: the transmitted waves may have effects on physiology of pain receptor and also, through microtrauma, they may initiate healing processes by the release of molecular agents and growth factors leading to neovascularization [21]. Despite increasing use of ESWT in the treatment of Plantar Fasciitis, few well-controlled trials have been conducted to approve its efficacy.

Considering the potential complications of both steroid injection and surgical treatment after conservative therapy, ESWT is a therapy that can be easily tolerated by patients [22]. There is, as of yet, no standard procedure for ESWT in the treatment of PF, such as intensity of energy, number of pulses, frequency of sessions and mode of administration. In different applications, the dose changes between 0.02 mJ/mm2 and 0.36 mJ/mm2 and the number of pulses changes between 1 000 and 4 000. It has been reported that a single session is performed at high energy intensity and three sessions are performed at moderate-low energy intensity [1].

In the present study, a total of three sessions of ESWT was performed at a dose of 2 000 pulse/ sessions in one-week intervals. Success rate of ESWT in the treatment of PF ranges from 57% to 88% [1]. Aqil et al. conducted a meta-analysis of seven different randomized controlled trials including a total of 663 participants (369 in the placebo group and 294 in the ESWT group) to investigate the efficacy of ESWT in the treatment of chronic PF [23]. They suggested that ESWT was an effective therapy as compared with the placebo and could be used in patients not benefiting from conservative therapy. In the present study, pain and functional status were also significantly improved in the patients receiving ESWT.

There is a limited number of studies in the literature comparing PRP and ESWT in the treatment of PF. In a study by Chew et al. conducted on 54 patients with PF, they determined that PRP injection and ESWT were superior to conventional therapy (stretching exercises of the Plantar fascia) in terms of pain and functional status during the 1st, 3rd and 6th months of follow-up, with no significant difference between the two methods [24]. Li et al. conducted a meta-analysis of 41 studies comprising a total of 2 889 patients and evaluated the efficacy of 8 different treatment methods used to treat PF (NSAIDs, corticosteroid injection, autologous blood transfusion, PRP injection, ESWT, ultrasound therapy, botulinum toxin A, and dry needling) in terms of VAS scores. They found that only ESWT was superior to placebo in terms of VAS score at the 1st month and that ESWT was again beneficial at the 3rd month. Accordingly, they concluded that ESWT was the optimum therapeutic method in PF patients, whereas botulinum toxin A and PRP injection might be suboptimal therapeutic methods [25].

The present study has some limitations:

The lack of a placebo-controlled group, evaluating the patients based on patient-reported outcomes alone (i.e., function and pain scores), and a relatively short follow-up period could be considered as the limitations of the present study.

In conclusion:

The functional scores significantly increased and the pain scores significantly decreased at the third month, as compared with the pretreatment (baseline) scores in both groups. Comparing the two groups, changes in the functional and pain scores were significantly better in the PRP group than in the ESWT group. None of the patients in either group developed complications. The

present study is one of a limited number of studies comparing PRP with ESWT in the treatment of PF: it is obvious that further large-scale studies are needed to compare the efficacy of these two methods.

Conflict of interests: The authors declare that there is no conflict of interests.

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Evaluation of scrotal pigmentation in infants with spectroscopic method and its correlation with 17-hydroxyprogesterone blood levels

Yenidoğanlarda skrotal pigmentasyonun spektrometre ile değerlendirilmesi ve 17-hidroksiprogesteron kan düzeyi ile korelasyonunun incelenmesi

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ABSTRACT

Aim: To evaluate the scrotal melanin density in infants using spectrometry and to determine the correlation between spectrometric evaluations, physical examinations and blood 17-hydroxyprogesterone levels.
Material and methods: A total of 40 infants were enrolled to the study, 22 of whom were diagnosed by a physician as having scrotal hyperpigmentation and 18 with normal scrotal pigmentation, who were admitted for the evaluation of prolonged jaundice. Age, gestational week, birth weight and scrotal pigmentation noted by the physician were recorded. Spectral data were acquired from scrotum and thigh. A correlation between the spectral measurements and the blood 17-hydroxyprogesterone level was determined by comparing spectral value in the wavelength range of 620-800 nm and 17-hydroxyprogesterone levels.
Results: No statistically significant difference was observed between the groups who were categorized by the physician as having "hyperpigmented" or "normal" scrotal color in terms of the infant's age, gestational week, birth weight, 17-hydroxyprogesterone level or spectrometric values. We observed a strong correlation between 17-hydroxyprogesterone levels and spectrometric values in all groups.
Conclusion: This preliminary study is the first one in the literature which evaluates scrotal pigmentation with an objective spectrometric method and determines its relationship with 17-hydroxyprogesterone levels. Further studies are needed to employ this method as a non-invasive, indirect screening test for the screening of congenital adrenal hyperplasia in male infants.

Key words: Congenital adrenal hyperplasia, spectrometry, scrotal pigmentation, 17-hydroxyprogesterone, spectroscopic method

ÖZ

Amaç: Spektrometre ile bebeklerin skrotal melanin dansitesini değerlendirmek ve spektrometrik değerlendirme ile fizik muayene ve kan 17-hidroksiprogesteron düzeyleri arasındaki ilişkiyi belirlemektir.
Gereç ve Yöntemler: Çalışmaya skrotal hiperpigmentasyonu olduğu düşünülen 22 olgu ile uzamış sarılık nedeniyle başvuran normal skrotal pigmentasyona sahip 18 olgu olmak üzere toplam 40 bebek dahil edildi. Olguların yaşı, gestasyon haftası, doğum ağırlığı, anne yaşı, skrotum rengi kayıt altına alındı. Spektral veriler skrotumdan ve uyluktan elde edildi. Spektral ölçümler ve kan 17- hidroksiprogesteron seviyesi arasındaki korelasyon, 620-800 nm dalga boyu aralığındaki spektral değer ve 17- hidroksiprogesteron seviyelerinin karşılaştırılmasıyla incelendi.
Bulgular: Skrotal hiperpigmentasyonu saptanan ve saptanmayan grup arasında anne-olgu yaşı, gestasyon haftası, doğum ağırlığı, 17-hidroksiprogesteron düzeyi ve spektrometri değeri arasında istatistiksel olarak anlamlı farklılık saptanmadı. Tüm gruplarda 17-hidroksiprogesteron düzeyleri ile spektrometrik değerler arasında güçlü bir korelasyon gözlemledik.
Sonuç: Çalışmamız, literatürde spektrometre değerleriyle skrotal pigmentasyonun nesnel bir ölçüt hale getirildiği, 17-hidroksiprogesteron ile ilişkisinin incelendiği, gelecekte erkek bebeklerde konjenital adrenal hiperplazi taramasına dolaylı yoldan yardımcı olabilecek bir çalışmadır.

Anahtar kelimeler: Konjenital adrenal hiperplazi, spektrometre, skrotal pigmentasyon, 17-hidroksiprogesteron, spektroskopik yöntem

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an autosomal-recessive inherited disease, resulting from deficiency of any of the 7 enzymes required for cortisol biosynthesis from cholesterol [1]. 21-hydroxylase enzyme deficiency accounts for more than about 95% of all CAH cases [2]. Impaired production of cortisol, aldosterone and increased androgen synthesis are the causes of many clinical manifestations.

While female infants with CAH are identified early due to their ambiguous genitalia, male infants do not have this sign to alert the physician. This results in diagnostic delays and diagnosis can only be made when serious adrenal insufficiency develops. Therefore, attention by the physician, particularly in the case of male infants, is very important for early diagnosis of the disease. Genital hyperpigmentation developing as a result of the disease in male infants is recognized as a warning sign [3]. In clinical practice, 17-OHP blood level is required in infants with suspected genital hyperpigmentation. However, this subjective criterion can lead to missing the cases or unnecessary 17-OHP requests.

Spectroscopic methods have begun to be widely used in recent years for the diagnosis of diseases. The objective of these studies is to diagnose the disease in a non-invasive and less pain-inflicting manner at an early stage. In addition, another aim of the researches made with optical methods is to develop practical systems that will enable the real time evaluation of the tissues [4, 5]. Spectroscopy is a non-invasive technique that can determine optical properties in vivo. In this technique, light is sent to the tissue and collected after interacting with it, then analyzed via the spectrometer. The spectrum data contains information about the biochemical composition and the physical structure of the tissue, thus providing information about the tissue physiology [6, 7].

The aim of this study was to develop an objective and non-invasive spectroscopic method to measure the density of melanin pigment in neonatal scrotal tissue and investigate the compliance of the method with physician's opinion and its correlation with 17-OHP blood level.

MATERIAL AND METHODS

Patients

This prospective study was conducted at the well-child clinic of Social Pediatrics Department at Akdeniz University. Twenty-two male patients older than two days of age thought to have scrotal hyperpigmentation on physical examination and 18 male patients aged 15-60 days with normal scrotal pigmentation on physical examination, who were assessed due to prolonged jaundice, were included in the study. Female infants and male infants with a nevus in the genital area and infants with low birth weight were excluded from the study. The age, gestational week and birth weight of the study infants were recorded. The physical examination of all cases was performed by the same physician and the scrotum color was recorded as either hyperpigmented or normally pigmented.

Spectroscopy System

The spectroscopy system consisted of a miniature spectrometer (Ocean Optics USB200, FL), an optical fiber probe to deliver the light to and from the scrotum (R400-7-VIS-NIR, Ocean Optics, FL), a white light source (HL-2000, Ocean Optics, FL) and a laptop computer. The optical fiber probe was made up of seven optical fibers with a core diameter of 400 µm; one of the fibers was placed at the center and the other six surrounded it. The six surrounding fibers delivered the light to the tissue and the central fiber detected reflected diffuse light from the tissue. A schematic illustration of the system is given in Figure 1.

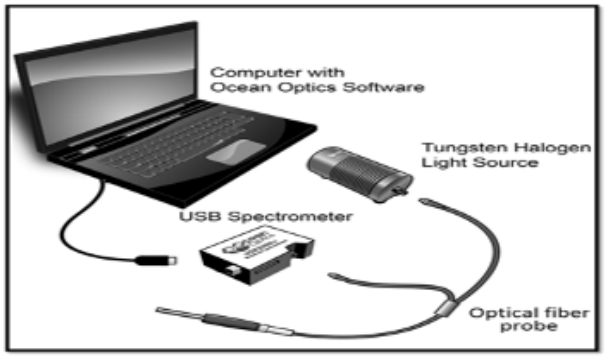

Figure 1. Schematic illustration of the spectroscopic system used in the study.

Measurements:

The necessary institutional and ethical approvals were obtained from Akdeniz University Ethics Committee (11.06.2013-115) at the beginning of the study in accordance with the Helsinki declaration. All parents gave written informed consent before participation.

The tip of the optical fiber probe was gently placed over the scrotum for the spectroscopic measurements. Before acquiring data from the scrotum, the system was calibrated as previously described [8]. Control measurements were made from the inside of the thigh to remove the effect of any components other than melanin, which are absorptive of light, located in the scrotum tissue.

The blood level of 17-OHP was determined via the RIA (DIAsource 17OH-RIA-CT Kit, DIAsource ImmunoAssays S.A., Belgium) method in all infants who had scrotal hyperpigmentation or normal pigmentation, according to the physician's evaluation. To avoid unnecessary blood sampling, infants with normal scrotal pigmentation were chosen among those who were scheduled for blood tests due to prolonged jaundice.

Data Analysis:

All the spectra were acquired in the wavelength range of 400-800 nm. The first spectrum was the background spectrum ($I_b(\square)$), acquired from the region of interest while the light source was off. The second spectrum ($I_s(\square)$) was taken to define the spectral distribution of the light source, with the probe placed nearly 1 cm above a white reflectance standard (Spectralon; Labsphere Inc, North Sutton, NH). Then, the spectra ($I_p(\square)$)were acquired from the patient's scrotum and medial side of the thigh. The spectra acquired from the patients were corrected as

$$_c(\lambda) = \frac{I_p(\lambda) - I_b(\lambda)}{I_s(\lambda) - I_b(\lambda)}$$

As seen in Figure 2, normalized spectra of scrotum and thigh are different from each other. Hemoglobin absorption is stronger on the thigh than the scrotum in the wavelength range of 500-600 nm. In all probability, the probe was located on a vein during the acquisition of the spectra from the thigh. Absorption of hemoglobin decrease above 600 nm. In the wavelength range of 620-800 nm, absorption of water is very weak, absorption of hemoglobin is lower than the absorption of melanin. Therefore, in that wavelength the range spectral shape is dominated by the absorption of melanin [9]. Basically, spectral shape of the transmitted light through the tissue depends on the tissue optical parameters defined by the absorption and scattering of the light within the tissue. Scattering depend on the physical structure of the tissue. Absorption depends on the concentration of each tissue chromophores. The difference between the thigh and the scrotum tissues in terms of the spectroscopy is the variation of melanin concentration. Scrotum tissue has more melanin than thigh tissue. Here, we assume that all other optical parameters of both tissues are similar except for the absorption of melanin. Therefore, having the ratio of the spectra provides the variation of melanin absorption in the wavelength range of 620-800 nm.

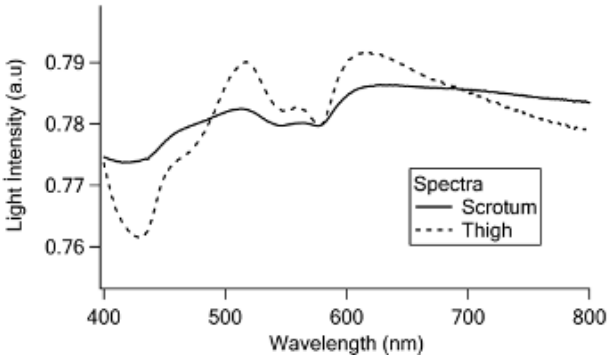

Figure 2. The spectrum measured from the scrotum and thigh: scrotum (solid line) and thigh (dashed line).

In order to obtain net absorption of the scrotum pigmentation, the scrotum spectrum is divided by the spectrum of normal tissue (the spectrum acquired from the thigh). As seen in Fig 3, the ratio is increasing with the wavelength. Slope of the ratio in the wavelength range of 620-800 nm has been chosen as a diagnostic parameter and calculated for all the patients. From this point on, we identify the "slope" as the "spectroscopic value". The correlation between the spectroscopic value and 17-OHP blood level was examined.

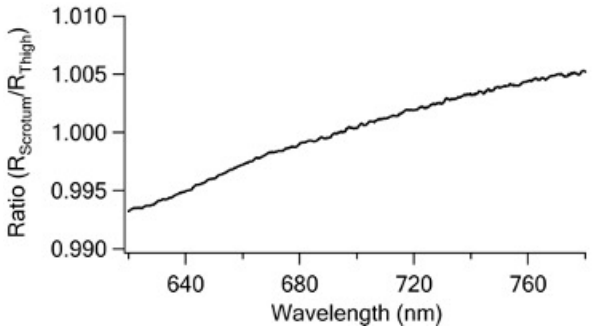

Figure 3. Ratio of the spectrum acquired from the scrotum to the spectrum acquired from thigh.

Statistical Analysis:

Data results were analyzed using the SPSS 18.0 (Chicago) software package. Descriptive statistics such as frequency distribution, mean and standard deviations were used to describe the sample. The Shapiro-Wilk normality test was used to analyze the continuous distributions of the two groups, Student-t test was performed in the cases with normal distribution, and Mann-Whitney U test was performed in the cases where non-conformity was observed. The Spearmann correlation analysis was used for the relationship analysis of the measurement variables according to the test assumptions. A 95% significance level (or α = 0.05 error margin) was used to determine the differences in the analyses. The following assumptions were made: a Spearman's correlation coefficient (r) between 0.90-1.00 indicates a very strong correlation, 0.70-0.89 a strong correlation, 0.50-0.69 a moderate correlation, 0.30-0.49 a weak correlation, 0-0.29 no correlation and a negative correlation indicates an inversely proportional correlation.

RESULTS

Physician-diagnosed scrotal hyperpigmentation was determined in 22 of the 40 infants studied. The median age of the infants was 29 (3-60) days, the mean birth weight was 3348 ± 387 grams, the mean gestational week was 38.1 ± 1.2 weeks, the mean spectroscopic value was 66.1x10-6 ± 29.5x10-6 and the mean 17-OHP level was 6.98 ± 3.8 ng /dl. The 17-OHP level of two cases was in the range of 10-20 ng/dl. The 17-OHP level of one case was above 20 ng/dl. Nine cases (22.5%) were born at 37 weeks' gestation or less.

When infants were grouped according to the physician's evaluation, as having normal or hyperpigmented scrotum, no statistically significant difference was observed between the two groups in terms of age, birth weight, gestational age, spectroscopic value or 17-OHP levels (Table 1).

Table 1. Demographic data, spectrometric measurement values and 17-OHP levels of infants with normal or hyperpigmented scrotum according to the physician's evaluation

	All Patients	Hyperpigmented (n:22)	Normal (n:18)	p value
Age (day)	20.8±11.97	22.4±14,1	18.9±8.8	.436
Birth weight (gram)	3348±387	3355±396	3340±386	.849
Gestational age (week)	38.1±1.2	38.3±1.0	37.9±1.3	.518
Spectroscopic value (x10-6)	66.1±29.5	69.8±31.9	61.6±26.6	.377
17 OH Progesterone level (ng/dl)	6.98±3.8	7.19±4.3	6.72±3.1	.807

Data are presented as mean ± SD.

In infants with physician-diagnosed scrotal hyperpigmentation, the gestational week had a weak positive correlation with birth weight (r=0.488, p=0.021). There was a weak negative correlation between the 17-OHP level and the age of the infant (r = -0.434, p = 0.044). A strong positive correlation was found between the 17-OHP level and the spectroscopic value (r=0.869, p=0.000).

In infants with scrotal pigmentation determined to be normal according to the physician's evaluation, the age and gestational week were not correlated with any of the parameters. The birth weight was negatively correlated with the 17-OHP level (r=-0.609, p=0.007). A strong positive correlation was found between the 17-OHP level and the spectroscopic value (r=0.837, p=0.000).

In all infants, the gestational week was found to have a weak positive correlation with the birth weight only (r=0.325 p=0.041). The birth weight had a weak negative correlation with the 17-OHP level (r=-0.332, p=0.036), whereas age of the infant was not correlated with any of the parameters. A strong positive correlation was found between the 17-OHP level and the spectroscopic value (r=0.857, p=0.000) (Figure 4).

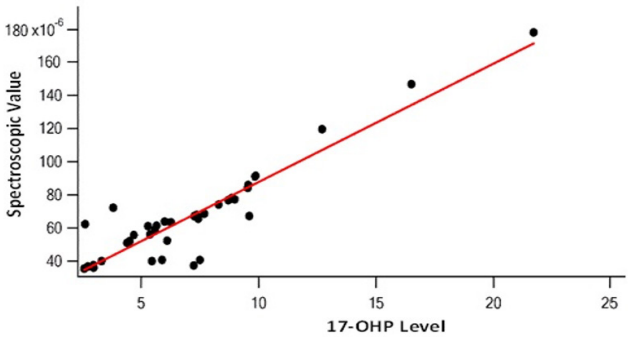

Figure 4. The relationship between 17-OHP level and spectroscopic value.

DISCUSSION

CAH is a common and fatal childhood disease, the morbidity and mortality of which can be prevented with early diagnosis and treatment [10]. The cost of screening for 21-hydroxylase deficiency in newborns is high. However, when the results obtained from the screenings were evaluated, at least 50% of the patients identified during the screening were not clinically diagnosed [11]. Therefore, attention on the part of the physician, particularly in the case of male infants, is very important for early diagnosis of the disease, in particular in countries where there exists no screening program for CAH. Genital hyperpigmentation developing as a result of the disease in male infants is recognized as a warning sign, however the subjective evaluation of physician may lead to false-positive suspicion which may in turn result in unwarranted anxiety for the parents and higher health care costs for the system. On the other hand, false-negative evaluations on physical examinations may lead to more devastating results such as late diagnosis, severe dehydration and even death. Thus, in our study, we tried to develop an objective method to measure the density of melanin pigment in neonatal scrotal tissue. We identified a strong positive correlation between the spectrometer value and the 17-OHP level in all infants, though there was no difference in the 17-OHP values when physician was the source of information about the pigmentation of scrotum. Hence, we believe that the spectrometric method may allow the determination of CAH in a more objective manner.

According to our research, this study is the first in the literature in which scrotal pigmentation was evaluated objectively with a non-invasive spectroscopic method and its correlation with the 17-OHP was investigated. We have shown that the spectrum of the light reflecting from scrotal tissue was strongly correlated with the 17OHP levels of infants (r = 0.857). We think this method can provide an initial, non-invasive, indirect step for the screening of CAH in the future for countries which do not a have a well-established CAH screening program. Consequently, we identified several major advantages: First of all, this spectroscopic method is portable and allows the probe to be easily positioned on the scrotum due to its flexibility. Secondly, patients are examined in a non-invasive manner by the optical fiber probe. Thirdly, this method is not motion sensitive during data acquisition. Fourthly, the system is completely safe for the patients because only visible light is used in the measurements. Lastly, acquiring a spectrum from scrotum takes less than 2 minutes and therefore allows the physician to diagnose scrotal hyperpigmentation in a short time.

The most important problem with screening tests is false positives at the 17-OHP level. The latter is high during birth and rapidly decreases in the next few days. In this respect, blood samples should be taken 48-72 hours after birth, making it difficult to diagnose during this period. Therefore, in order to eliminate the effect of blood sampling on the 17-OHP level, we exclusively included infants aged 2 and more days in the study.

Preterm, sick or stressed infants have higher 17-OHP levels than healthy children. Especially in preterm infants, physiological delay in 11 β-hydroxylase enzyme activity can cause temporary increases in 17-OHP levels [12]. Van der Kamp et al. [13] reported that 17-OHP levels are correlated more with gestational week than birth weight. In our study, we found a weak negative correlation between the 17-OHP level and the birth weight (r=-0.332) but we did not find any significant correlation between the gestational week and the 17-OHP level (p=0.456). This may be ascribed to the exclusion of very young preterm infants and infants with low birth weight from the study.

In our study, we did not find any significant

difference between 17-OHP levels of patients with scrotal hyperpigmentation and those without, as revealed by the physical examination (p=0.807). We think this is important because it shows that the physician may fail to correctly diagnose CAH. However, we found a strong positive correlation between the 17-OHP level and the spectroscopic value of all patients (r=0.857, p=0.000).

As a matter of fact, Gökdemir et al. [14] found scrotal hyperpigmentation in 90 (28.12%) of 320 male infants they included in their study. This percentage indicates that the incidence of scrotal hyperpigmentation in male infants should not be underestimated and that the evaluation of scrotal hyperpigmentation through physical examination is a subjective practice that is not very helpful in diagnosis of CAH, frequently giving false positive results. This, in turn, leads to additional unnecessary interventional procedures.

The limitations of our study were that we did not examine the correlation of spectroscopic value with the 17-OHP in patients with low birth weight, and that there were no diagnosis of CAH among our patients. Therefore, the lack of a control group is a limitation of the study and this is the reason we could not provide a cut-off value for spectroscopic density in this preliminary study.

In this pilot study we observed that the 17-OHP blood levels correlated with the spectroscopic values, but not with the scrotal hyperpigmentation identified by physical examination. In order for spectrometry to be used as an indirect and non-invasive method in diagnosis of CAH, further studies are required to determine the sensitivity and specificity of the test, by comparing the spectrometric cut-off value corresponding to 10 ng/ml of the 17-OHP blood level in two large patient groups, with and without CAH diagnosis.

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Conflict of Interest: The authors have no conflicts of interest relevant for this article.

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Evaluation of ezrin and fascin 1 in the PFOS treated Sertoli cell culture: An in vitro study

PFOS ile tedavi edilen Sertoli hücre kültüründe ezrin ve fascin 1'in araştırılması: İn vitro bir çalışma

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ABSTRACT

Aim: Depending on the findings resulting from the knock-downing of ezrin and of fascin 1 in vivo, we aim to show the defects or disruption of the blood-testis barrier (BTB) structure and F-actin bundling after Perfluorooctanesulfonate (PFOS) treatment in primary Sertoli cell culture.

Study Design: Primary Sertoli cell isolation was occurred with control and PFOS-treated (20M) groups. Sertoli cells were prepared for both experiments as 0.5 x 106 cell/ml.

Methods: Dual-labeled immunofluorescence analysis to assess co-localization of fascin 1 with ezrin both in Sertoli cells was performed, and Co-IP, by using lysates of seminiferous tubules, was performed using actin and ezrin proteins to identify specific protein-protein interaction with fascin 1.

Results: Firstly, we showed that ezrin and fascin 1, which were components of the ectoplasmic specialization were co-localized in the Sertoli cells and also they were interacted each other. Secondly, we indicated that they were dislocated in the PFOS-treated Sertoli cells in vitro. Because of PFOS (20M), the actin-based cytoskeleton was no longer capable of supporting the distribution and/or localization of actin-regulatory proteins at the cell-cell interface necessary to maintain localization of actin-regulatory at the BTB.

Conclusion: In summary, these findings suggest that ezrin and fascin 1 can work together to preserve BTB integrity by regulating F-actin organization in the PFOS-mediated Sertoli cell disruption.

Keywords: Blood-testis barrier, ectoplasmic specialization, F-actin, ezrin, fascin 1,

ÖZ

Amaç: Bu çalışmada, Ezrin ve Fascin 1'in in vivo olarak baskılanması sonucundaki bulgularla birlikte, Kan testis bariyeri (KTB) yapısının bozulması ve primer Sertoli hücre kültüründe, Perflorooktansülfonat (PFOS) muamelesinden sonra F-aktin demetlenmesinin gösterilmesi amaçlanmaktadır.

Çalışma Tasarımı: Primer Sertoli hücre izolasyonu, kontrol ve PFOS ile muamele edilen (20μM) gruplarla yapıldı. Sertoli hücre konsantrasyonu her iki deney için 0.5 x 106 hücre/ml olacak şekilde hazırlandı.

Metod: Hem Fascin 1'in Sertoli hücrelerinde Ezrin ile birlikte lokalizasyonunu değerlendirmek üzere çift etiketli immünofloresan analizi yapıldı, hem de seminifer tübül lizatlarından aktin ve Ezrin proteinlerinin Fascin 1 proteini ile spesifik protein-protein etkileşimini tanımlamak için Co-IP deneyi uygulandı.

Bulgular: İlk olarak, ektoplazma özelleşmesi bileşenleri olan Ezrin ve Fascin 1 in Sertoli hücrelerinde ko-lokalize olduğu ve birbirleri ile etkileşime girdiği gösterilmiştir. İkinci olarak ise PFOS ile muamele edilen Sertoli hücrelerinde Ezrin ve Fascin 1 dislokasyonu gösterilmiştir. 20μM PFOS uygulandığında aktin tabanlı hücre iskeleti KTB'deki hüce-hücre bağlantı bölgelerindeki aktin düzenleyici proteinlerin yayılmasını ve/veya lokalizasyonunu destekleyememiştir.

Tartışma: Sonuç olarak bu bulgular, PFOS aracılı Sertoli hücre bozulmasında, Ezrin ve Fascin 1'in KTB bütünlüğünü korumak için F-aktin organizasyonunu düzenleyerek birlikte çalışabileceğini desteklemektedir.

Anahtar Kelimeler: Kan-Testis Bariyeri, Ektoplazmik Özelleşme, F-actin, ezrin, fascin

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INTRODUCTION

Studies in the last decade using the blood-testis barrier (BTB) in rats have demonstrated the presence of several signaling pathways [1]. Morphological details of germ cell transport occurs through actin-based cytoskeleton formation during spermatogenesis in rodents [2]. BTB remodeling is important to understand germ cell transport through the actin-based re-bundling mechanism [3]. Ezrin leads actin microfilaments in spermatogenesis to integral membrane protein, as well as peripheral protein in mammalian cells, to organize apical membrane domain, which create a scaffold for signaling molecules to regulate cell migration, proliferation, adhesion, and polarity [4, 5]. A knockdown of ezrin in vivo by RNAi was found to impede spermatid transport, causing defects in spermiation in which spermatids were embedded deep inside the epithelium, and associated with a loss of spermatid polarity [6]. Fascin is a 56-kDa polypeptide, possessing the actin binding and bundling activity by cross-linking filamentous actin into tightly packed parallel bundles [7, 8]. A knockdown of fascin 1 in vivo by 60–70% induced defects in spermatid polarity, which was mediated by a mislocalization and/or downregulation of actin-bundling proteins, impeding F-actin organization and disrupting spermatid polarity. Perfluorooctanesulfonate (PFOS), served as a fabric protector and an active component in stain repellents [9] by 3M in 1949, as a fluorosurfactant. Its toxic effects have begun to emerge, initially detected in wildlife and then in humans, and they include defects in development, cancer, endocrine disruption, neonatal mortality [10, 11], and an increased risk of attention-deficit hyperactivity disorder [12]. PFOS was also found to induce Sertoli cell injury by perturbing actin cytoskeleton through changes in the spatial expression of actin regulatory proteins [13] and the organization of F-actin in Sertoli cells [14]. With this study we aim to investigate how PFOS affects ezrin and fascin 1 expression pattern and prevent the organisation of F-actin bundling in BTB.

MATERIAL AND METHODS

Animals

Sprague-Dawley (outbreed) rats were euthanized by CO_2 asphyxiation using slow (20–30%/min) displacement of chamber air with compressed carbon dioxide, in a euthanasia chamber (Braintree Scientific, Braintree, MA). Experimental protocol was approved by animal care and usage committe of Istanbul University and was in accordance with the institiuational Animal Care and Use Committee (IACUC) guide.

Primary Sertoli cell cultures

Sertoli cells were isolated from 20-day-old rat testes and cultured for experiments reported herein, as detailed elsewhere [6]. Cells were cultured in serum-free Ham's F-12 Nutrient Mixture-Dulbecco's modified Eagle's medium (F-12- DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with bovine insulin, human transferrin, EGF, bacitracin, and gentamicin in a humidified CO_2 incubator with 95% air-5% CO_2 (vol/vol) in a humidified atmosphere at 35°C. After isolation, Sertoli cells were plated on Matrigel (BD Biosciences, Billerica, MA)-coated coverslips, 12-well culture dishes, 0.5, and 1.2 x 106 cells/cm2. Cells at these densities were used for the following corresponding experiments: 1) dual-labeled immunofluorescence analysis, including F-actin staining; 2) lysate preparation for immunoblotting and Co-IP.

Toxicants

PFOS (perfluorooctanesulfonic acid, potassium salt, Mr 538.22, Sigma-Aldrich) was dissolved in dimethylsulfoxide (DMSO) as a 100- to 200-mM stock so that the concentration of DMSO in Sertoli cell cultures was approximately 0.02% (vol/vol). PFOS administration used between 3, 6, 24 and 48 hours in primary Sertoli cell culture.

Dual-labeled immunofluorescence analysis

Immunofluorescence analysis using cultured Sertoli cells was performed as described [15]. Primary antibodies for Ezrin (Abcam cat no: ab4069, 1/200 dilution), fascin 1 (Abcam cat no: ab126722, 1/100) , F-actin (Phalloidin, sigma, p5282, 1/150 dilution) used for this study. Secondary antibodies were goat antimouse or goat antirabbit IgG conjugated to either Alexa Fluor 488 or 555 (Invitrogen) and diluted 1:200 in PBS containing 1% BSA (vol/vol). Cells were mounted in ProLong antifade reagent containing

4′,6-diamidino-2-phenylindole (DAPI; Invitrogen), and images were acquired with MicroSuite FIVE software (version 1.224; Olympus Soft Imaging Solutions Corp) using an Olympus DP71 12.5 MP (megapixel) digital camera in an Olympus BX61 motorized fluorescence microscope (Olympus America, Inc). All samples within experimental set vs. controls were processed and analyzed in a single experimental session to avoid inter-experimental variations. A representative set of data was shown herein, but each experiment was repeated at least three times using different preparations of Sertoli cells with similar findings.

Immunoblotting and Co-IP.

Lysates were obtained from Sertoli cells and seminiferious tubules. Tubules isolated from adult rat testes were used within 2 hours after their isolation, which were devoid of Leydig cell contamination. Actin (sc-1616) antibody used for immunoblotting for Co-IP with 1/300 dilution. Co-IP was performed using lysates (around 600 µg protein) from testes or tubules. Chemiluminescence was performed and the immunoblots were analyzed as described [16]. Immunoblotting data were acquired in a Fujifilm LAS-4000 Mini Imaging System and analyzed in MultiGauge software (version 3.1; Fujifilm), which was then quantified by using the Scion Image software package (version 4.0.3.2, Scion; http://scion-image.software.informer.com/) for analysis, as described [17].

Statistical analysis

Significance was determined by one-way ANOVA, followed by Dunnett's procedure using the SigmaStat software package (version 3.5; Systat Software Inc). *; p >0.05.

RESULTS

Ezrin and fascin 1 expression patterns in Sertoli cells together with their co-immunuprecipitation levels.

We first examined fascin 1 and ezrin expression levels in Sertoli cells (Figure 1A). We showed that ezrin and fascin 1 expressions were co-localized in Sertoli cell cytoplasm with high expression levels. We next observed that ezrin and fascin 1 were co-immunoprecipitated together with actin protein (Figure 1B). We found that anti-fascin-1 IgG was labeled with ezrin expression pattern in seminiferous tubule (ST) lysates.

PFOS perturbs F-actin organization at the BTB, impeding the localization and/or distribution of Ezrin and F-actin at the Sertoli cell-cell interface

PFOS induced changes in the localization of ezrin and distribution of F-actin in the Sertoli cell epithelium by dual-labeled immunofluorescence (Figure 2). It perturbed the organization of F-actin in Sertoli cells. PFOS-treated (6h) Sertoli cells, actin filaments in cell cytosol were truncated and defragmented and actin filaments no longer assumed the orderly bundled configuration, which was not present in the control Sertoli cells (Figure 2.).

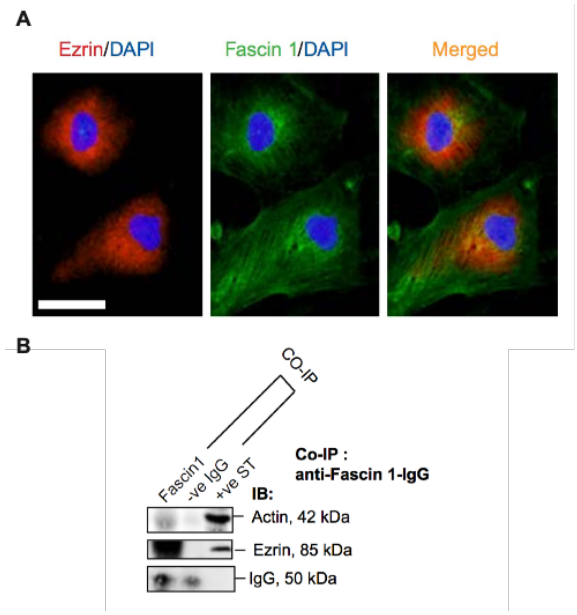

Figure 1. (A) Dual-labeled immunofluorescence analysis to assess colocalization of fascin 1 (green fluorescence) with ezrin (red fluorescence) in Sertoli cells. Sertoli cells nuclei were visualized by DAPI. Scale bar, 15 mm (applies to all micrographs.) (B) Co-IP using lysates of seminiferous tubules was performed using actin and ezrin proteins to identify specific protein-protein interaction with fascin 1. IgG, heavy (50 kDa) chains, served as the protein loading control.

PFOS perturbs F-actin organization at the BTB, impeding the localization and/or distribution of fascin 1 and F-actin at the Sertoli cell-cell interface

PFOS induced changes in the localization of fascin 1 and F-actin in the Sertoli cell epithelium, which were demonstrated by dual-labeled immunofluorescence staining (Figure 3). After PFOS treatment, the expression levels of fascin 1 together with F-actin were affected and these

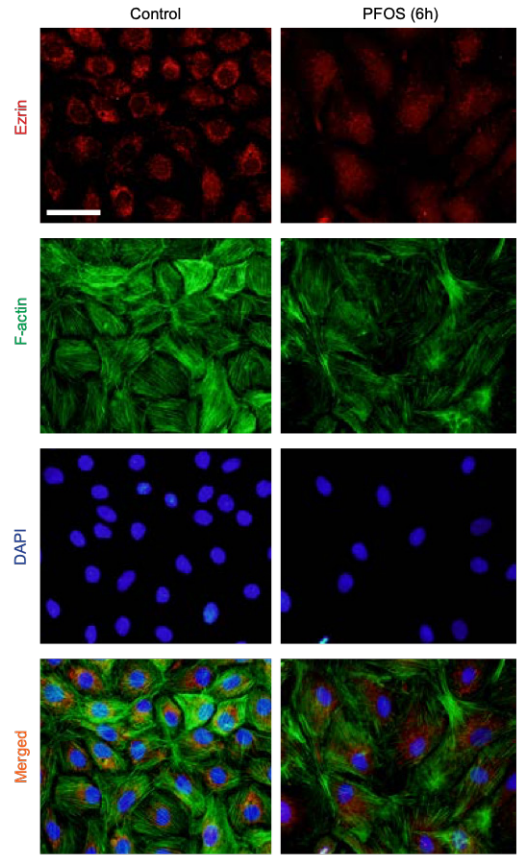

Figure 2. Dual-labeled immunofluorescence analysis to assess colocalization of ezrin (red fluorescence) with F-actin (green fluorescence) in PFOS-treated Sertoli cells. Sertoli cell nuclei were visualized by DAPI. Scale bar, 15 mm (applies to all micrographs).

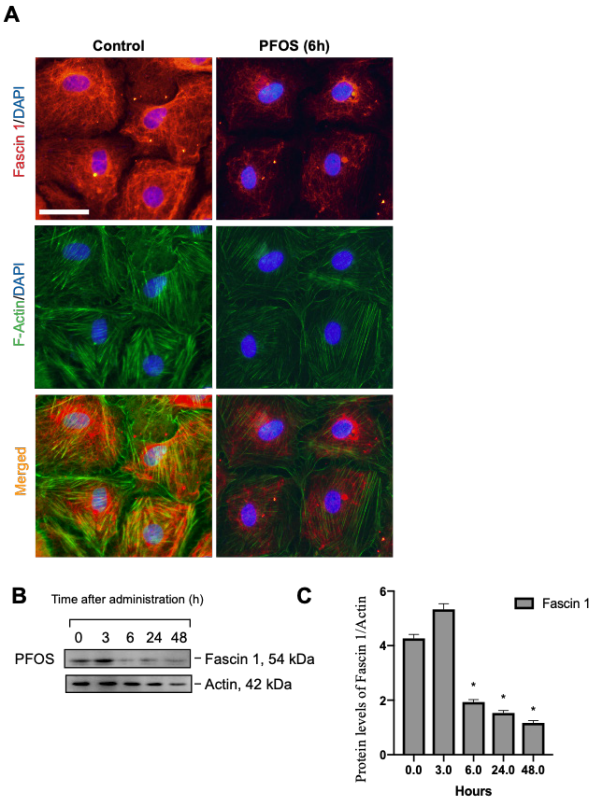

Figure 3. (A) Dual-labeled immunofluorescence analysis to assess colocalization of fascin 1 (red fluorescence) with F-actin (green fluorescence) in PFOS-treated Sertoli cells. Sertoli cell nuclei were visualized by DAPI. Scale bar, 15 mm (applies to all micrographs). (B) Fascin 1 expression levels after PFOS administration (0, 3, 6, 24, 48 hours) with actin as an internal control. (C) Protein levels of Fascin 1 immunoblotting data was analyzed by comparing 5 different hours with at least 3 Fascin 1 bands, which were normalized by actin protein levels.

proteins were mislocalized and their expression levels were decreased (Figure 3A). After PFOS administration in 3, 6, 24 and 48 hours we showed that Fascin 1 expression levels were decreased after 6 hours of PFOS treatment (Figure 3B).

DISCUSSION

Earlier toxicity studies on PFOS have focused mostly on its disruptive effects on thyroid function in humans [18, 19]. In our study, PFOS perturbs the BTB via its effects on F-actin organization related with ezrin and fascin 1 co-operation at the same time. In a recent study that investigated the disruptive effects of PFOS on ezrin and fascin 1 in Sertoli cells, PFOS was found to reduce cell viability by inducing Sertoli cell reactive oxygen species production dose dependently [20]. However, F-actin disruption at the BTB was not studied together with ezrin and fascin expression patterns [21]. Herein we report that PFOS is a potential disruptor for the BTB organization, which was related to ezrin and fascin 1 expression patterns after 6 hour administration. This disrupting effect appears to be mediated initially by changes in the expression and localization of actin bundling proteins ezrin and fascin 1 in Sertoli cells. We have already known that actin bundling proteins leads to regulate germ cell migration during spermatogenesis [22]. These changes, in turn, perturb F-actin organization at the BTB, rendering actin filament bundles, the hallmark ultrastructure of the BTB in mammalian testes [23], which are unable to assume their bundled configuration to support BTB integrity. Instead, actin filaments in PFOS-treated Sertoli cells were truncated and defragmented together with the decreation of ezrin and fascin 1 expression levels. In summary, the regulation of the mechanism of actin bundling in PFOS-treated Sertoli cells can be controlled by ezrin and fascin 1 at the same time by using

the same signalling pathway in BTB. Thus, the responsibility of actin regulating proteins have to be proven by investigating their co-operations in different suggesting toxic models [24, 25] for BTB.

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Antioxidant Effects of Myrtus communis L.'s Essential Oils in BEAS-2B Cells Induced by Oxidative Stress with Hydrogen Peroxide

Hidrojen Peroksit ile Okdisatif Stresin İndüklendiği BEAS-2B Hücrelerinde Myrtus communis L. Esansiyel Yağının Antioksidan Etkilerinin Araştırılması

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ABSTRACT

Aim: In this study, the effects of *Myrtus communis* L. essential oil on the human bronchial epithelial cell line (BEAS-2B) exposed to oxidative stress with hydrogen peroxide were investigated and their effects on apoptotic pathways.

Materials and Methods: The MTT 3- (4,5-Dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide method was used to determine the appropriate doses of hydrogen peroxide (H_2O_2) and *M. communis* L.'s essential oil in BEAS-2B cells. Oxidative stress formation was determined by measuring malondialdehyde (MDA) level. The cells were divided into three groups: the group exposed to oxidative stress (group with H_2O_2), the treatment group (H_2O_2 + *M. communis* L.'s essential oil) and the control group. MDA levels were measured in all three groups and expression levels of Caspase 3, Caspase 8, Caspase 9 and p21 genes were determined by RT-PCR method in order to detect apoptotic effects.

Results: According to MTT test results, the appropriate doses were 40 µM for H_2O_2 and 15.625 µg/ml for *M. communis* L.'s essential oil. MDA levels were significantly increased in the group treated with 40 µM H_2O_2 when compared with the healthy cell group (p=0.0005). In the group treated with essential oil of *M. communis* L., MDA level was found similar to the control group (p>0.05). Expression levels of Caspase 3, Caspase 8 and p21 genes were significantly increased in cells where H_2O_2 was adminisrated at 40 µM concentrations compared to healthy cell group (p=0.001, p=0.017 and p=0.0003, respectively). However, Caspase 9 gene expression level did not change significantly (p=0.8). Compared to the group in which the oxidative stress model was established, it was found that Caspase 3 gene expression level decreased significantly in the cells treated with *M. communis* L.'s oil (p=0.00007).

Conclusion: In our study, it was shown that the essential oil of *M. communis* L. strongly decreased MDA levels and also had the potential to be a therapeutic agent due to its apoptotic inhibiting effect. *M. communis* L. has a strong antioxidant effect and is thought to be effective in stopping apoptosis caused by oxidative stress.

Keywords: Myrtus communis L, Apoptosis, BEAS-2B, Oxidative Stress

ÖZ

Amaç: Bu çalışmada, Myrtus communis L. esansiyel yağının, in vitro olarak hidrojen peroksit ile oksidatif strese uğratılmış insan bronşiyal epitel hücre hattında (BEAS-2B) antioksidan etkilerinin olup olmadığı ve apoptotik yolaklar üzerindeki etkileri araştırılmıştır.

Materyal ve Metod: BEAS-2B hücrelerinde H_2O_2 ile *M. communis* L. esansiyel yağının uygun dozlarının belirlenmesinde MTT yöntemi kullanılmıştır. Oksidatif stres oluşumu MDA düzeyi ölçülerek belirlenmiştir. Hücreler; oksidatif strese maruz bırakılan grup (H_2O_2), tedavili grup (H_2O_2+*M. communis* L) ve kontrol grubu olmak üzere üç gruba ayrılmıştır. Her üç grupta MDA düzeyleri ölçülmüş ve apoptotik etkilerin saptanması amacıyla Kaspaz 3, Kaspaz 8, Kaspaz 9 ile p21 genlerinin ekspresyon düzeyleri RT-PCR yöntemi ile saptanmıştır.

Bulgular: MTT testi ile uygun dozlar H_2O_2 için 40 µM, *M. communis* L. yağı için 15.625 µg/ml olarak saptanmıştır. Sağlıklı hücre grubuyla karşılaştırıldığında, 40 µM H_2O_2 maruziyeti uygulanan grupta MDA düzeyinde anlamlı artış gözlenirken (p=0,0005), *M. communis* L. esansiyel yağı ile tedavi edilen gruptaki MDA düzeyi kontrol grubu ile benzer düzeyde (p>0.05) bulunmuştur. 40 µM H_2O_2 uygulanan hücrelerde Kaspaz 3, Kaspaz 8 ve p21 genlerinin ekspresyon seviyelerinin sağlıklı hücre grubuna göre anlamlı bir şekilde arttığı (sırasıyla p=0.001, p=0.017 ve p=0,0003), Kaspaz 9 gen ekspresyon seviyesinin değişmediği (p=0.8) saptanmıştır. *M. communis* L. yağı ile tedavi edilen hücrelerde, oksidatif stres modelinin oluşturulduğu gruba göre Kaspaz 3 gen ekspresyon seviyesinin anlamlı şekilde azaldığı saptanmıştır (p=0.00007).

Sonuç: Çalışmamızda, *M. communis* L. esansiyel yağının MDA seviyesini güçlü şekilde azaltıcı etkisi olduğu, ayrıca apopitozu durdurucu bir etki göstererek, teröpatik ajan olarak potansiyeli olduğu gösterildi. Bu bağlamda *M. communis* L. yağının güçlü bir antioksidan etkiye sahip olduğu, oksidatif stresten kaynaklanan apoptozisin durdurulmasında etkili olduğu düşünülmektedir.

Anahtar Kelimeler: Myrtus communis L, Apopitozis, BEAS-2B, Oksidatif Stres

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INTRODUCTION

Myrtle (*Myrtus communis* L) is an evergreen plant of the Myrtaceae family. It grows naturally in the Mediterranean region and has been used as food and spices since ancient times. In addition, medical use is also common. Its leaves and fruits have traditionally been used as antiseptic, disinfectant and hypoglycemic agents [1]. Murten, sturgeon, and especially in Hatay "hambeles" also known as *M. communis* L., especially Turkey, Greece, Italy, Algeria, Tunisia and Morocco are grown widely in Mediterranean countries [2]. In folk medicine, the fruit of the plant is used in the treatment of various infectious diseases, including diarrhea and dysentery. The leaves of the plant are used as antiseptic and anti-inflammatory agent in wound healing and treatment of candidiasis [3]. Oil composition of the plant varies according to geographical location. According to numerous published scientific studies, essential oil has a strong antimicrobial activity and is widely used in the cosmetics, pharmaceutical and food industries [4,5].

Oxidative stress, defined as the imbalance between the levels of various oxidant molecules and antioxidants, can often lead to biochemical changes and thus serious diseases in many organisms [6]. Oxidative stress can cause cytotoxic and genotoxic effects, while damaging basic biomolecules such as lipids, proteins and DNA [7]. Nowadays, the damages of mutagenicity and reactive oxygen species have been shown in many diseases such as aging, atherosclerosis, cancer, diabetes and various neurodegenerative disorders. It is clear that it takes place. Over the past two decades, natural antioxidants have been highly emphasized [8,9]. Many essential oils and their components have recently been described as natural antioxidants, and have been proposed as a potential alternative to synthetic antioxidants.

In this study, it was investigated whether M.communis L.'s essential oil suppresses induced apoptosis due to its antioxidant effects and oxidative damage in human bronchial epithelial cells (BEAS-2B) exposed to oxidative stress with hydrogen peroxide.

MATERIAL and METHODS

Cell Culture

In the study, the BEAS-2B cells, a human bronchial epithelial cell line derived from healthy bronchial epithelial tissue, were used. The cell line was obtained from the cell culture laboratory of Mustafa Kemal University Medical Biology Department. The BEAS-2B cell line is a Human Bronchial Epithelial Cell line widely used in in-vitro studies. The BEAS-2B cell line was cultured in Dulbeco medium containing 10% Fetal Bovine Serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in 175 cm^2 culture flasks. Incubation of the cells was performed in a 95% humidified atmosphere and 5% CO_2 at 37 ° C. The medium was changed twice a week to maintain the cultures. Cell culture studies were performed when the cells covered 70-80% of the culture flasks.

Preparation of *M. communis* L.'s Essential Oil Doses

The essential oil we used in the present study was obtained from the Faculty of Agriculture at Hatay Mustafa Kemal University. Doses of 1000-500-250-125-62.5-31.25-15.625 µg/ml *M. communis* L. were prepared by serial dilution (dissolving the highest dose in 1% DMSO) in serum-free DMEM medium. The MTT test was performed to determine the appropriate dose.

Preparation of H_2O_2 Doses

In order to determine the dose that can be applied, the commercially available stock H_2O_2 was diluted in serum-free DMEM medium. MTT test was performed for doses diluted 0, 10, 20, 30, 40, 50, 75 and 100 µM.

Cell Viability

Cell viability was analyzed by MTT test. For the MTT assay, DMEM medium containing 10% FBS and 1% penicillin/streptomycin in 48-well plates was inoculated to $1x10^5$ cells/ml. Incubation of the cells was performed in a 95% humidified atmosphere and 5% CO_2 incubator at 37 °C. The cells were expected to coat the culture vessel surface at 70-80%. Serum-free medium was then added to the cells and incubated under the same

conditions for 24 hours. Two different MTT tests were performed on the prepared cells for different concentrations of H_2O_2 and essential oil of *M. communis* L.

To determine the non-cytotoxic concentrations of *M. communis* L.'s oil, the cells were exposed to essential oil of *M. communis* L. at concentrations of 1000-500-250-125-62.5-31.25-15.625 µg/ml for 24 hours. Similarly, to determine non-cytotoxic concentrations of H_2O_2, the cells were exposed to doses of H_2O_2 of 0, 10, 20, 30, 40, 50, 75, 100 µM for 24 hours. For both MTT experiments: MTT solution was prepared to be 1 mg/ml. The solution in the 48-well plates was removed, and 250 µl MTT solution was added to each well. The plate was then incubated for 1 hour at 37 °C. At the end of the incubation period, the MTT solution in the plate was discarded and inverted on a napkin for 2-3 min. Then, 250 µl of DMSO was added to each well and the plate was incubated at room temperature for 5 min. The color change was evaluated with a spectrophotometer at 590-670 nm wavelength.

Oxidative Stress

Cultivation of BEAS-2B cells was performed in 175 cm^2 cell culture flasks. The cell density was adjusted to $1x10^5$ cells/ml. DMEM (Dulbecco's Modified Eagle Medium, Gibco, UK) containing 10% FBS (Gibco, USA) and 1% antibiotic suspension (penicillin/streptomycin; Gibco, USA) was used as cell maintenance medium. The incubation of the cells was continued until the cells covered 70-80% of the surface of the culture flasks. At the end of incubation, serum-containing medium in cell culture flasks was discarded, and serum and pyruvate-free medium was placed as culture medium. The cells were treated with 0.40 µM H_2O_2 and *M. communis* L.'s essential oil (15.625 µg/ml) + 40 H_2O_2. And evaluation was performed after 24 hours incubation. The cells were harvested from the culture vessel surface and collected in a separate tube in cold HBSS. The collected samples were stored at -80 °C until the study was performed. For the tests, the cells were thawed at -80 ° C, and then with a homogenizer medium for 30 min. homogenized, and MDA and protein measurements were performed. MDA levels were determined by the method described by Sushil et al. previously [10].

MDA, the final product of lipid peroxidation, forms a pink complex when incubated with thiobarbituric acid at 95 °C and pH 3.5 under aerobic conditions. MDA amount was determined by spectrophotometric measurement of this complex at 532 nm wavelength. By measuring the amount of protein in each sample, the MDA was calculated as nmol/mg protein.

Gene Expression

The transcription levels of Caspase 3, Caspase 8, Caspase 9, p21 genes and β-actin gene as reference gene were determined by real-time PCR method. The primer sequences of these genes are given in Table 1.

Statistical Analysis

MTT analysis results of the study were calculated by applying GraphPad Prism Version 5.01 (GraphPad Software Inc., USA) program. The suitability of the data in the groups for normal distribution was determined by Shapiro-Wilk test. Kruskal-Wallis test was used to determine whether there was a difference between the groups. Dunn's Multiple Comparison Test was used to determine the significance between the groups. Each study group was compared with the control group and values $p < 0.05$ were considered statistically significant. Results are given as mean (mean) ± standard deviation. Analysis of gene expression data was performed with RT2 profiler PCR Array Data Analysis version 3.5. β-actin was used as the "housekeeping gene". The results were given as Fold change.

RESULTS

Assessment of Cell Viability

Different concentrations of H_2O_2 (0, 10, 20, 30, 40, 50 and 75 and 100 µM) were used to determine the appropriate doses to generate an oxidative stress model in BEAS-2B cells. The cells were exposed to H_2O_2 for 24 hours, and then cell viability analysis was performed by the MTT method. The effect of H_2O_2 was compared to the control group (group not containing H_2O_2) after 24 hours incubation in BEAS-2B cells. In the experiments, no significant difference was observed in the viability of cells

exposed to H_2O_2 doses of 30 µM and lower, whereas 40, 50, 75 and 100 µM doses of H_2O_2 significantly reduced cell viability (Figure 1.A). Cytotoxic effect was detected at the strongest level at a dose of 100 µM ($p<0.0001$) (Table 2).

Table 1. Primer sequences of genes whose expression levels were determined.

Gene	Forward Primer	Reverse Primer
Caspase 3	5'-CTTCTACAAC-GATCCCCTCTG-3'	5'-TGTGCTTCTGAG-CCATGGGTG-3'
Caspase 8	5'-GGGCTCAATTTCT-GCCTAC-3'	5'-GGCAC TGGCT-GTTTGCTT-3'
Caspase 9	5'-GTCACAAGACCTT-GACACCCG-3'	5'-ACCAGGTG-GTCTAGGGGTTT-3'
p21	5'-CCGAAGT-CAGTTCCTTGTGG-3'	5'-AGTACGGCCA-GAGGTGTACG-3'
β-aktin	5'-TCAACACCCCAGC-CATGTA-3'	5'-AGTACGGCCA-GAGGTGTACG-3'

Table 2. The mean optical density ± standard deviation values of the applied hydrogen peroxide doses.

H_2O_2 µM	Mean ± Standard Deviation	p value
100 µM	0.04073±0.005347***	< 0,0001
75 µM	0.07079±0.01433***	< 0,0001
50 µM	0.1919±0.04240***	< 0,0001
40 µM	0.2323±0.04196*	0,0202
30 µM	0.2639±0.04529	0.4769
20 µM	0.3031±0.04686	0,9996
10 µM	0.3170±0.04720	0,8715
Control (0)	0.2966±0.06281	-

Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001.

In BEAS-2B cells, different concentrations of essential oils were studied (15,625-31,25-62,5-125-250-500-100 µg/ml) to determine the appropriate doses of *M. communis* L. for essential oil.

After the exposure of the essential oil of *M. communis* L. to the cells for 24 hours, the cell viability analysis was performed by MTT method. At the end of the 24-hour incubation, no significant difference was detected between the control group and the cell viability of the essential oil concentrations of 62.5 µg/ml and lower (Figure 1.B.) ($p> 0.05$). However, at doses of 125-250-500-1000 µg/ml of essential oil, it was found to be significantly lower in cell viability compared to the control group (Table 3).

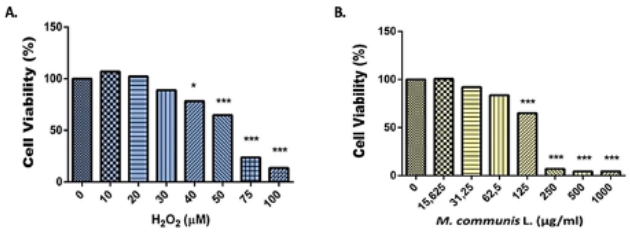

Figure 1. A. Cell viability graph of 24 hours H_2O_2 exposure at different doses in BEAS-2B cell line,
B. Cell viability graph of 24 hours M. communis L.'s essential oil exposure at different doses in BEAS-2B cell line (Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001).

In the following experiments, the lowest dose of H_2O_2 (40 µM) in which cell viability was suppressed and the lowest dose of essential oil of *M. communis* L. (15,625 µg/ml) in which the therapeutic effect was to be investigated were selected in order to detect oxidative stress stimulation. In all subsequent steps, the cells divided into 3 groups:

i) Control group (healthy cell group in which no substance was added)

ii) Oxidative stress-induced group (40 µM H_2O_2 added)

iii) Treatment group with *M. communis* L.'s oil (40 µM H_2O_2 + 15,625µg/ml essential oil of *M. communis* L. added).

Table 3. The mean optical density ± standard deviation values of applied *M. communis* L.'s essential oil doses.

M. communis L. µg/ml	Mean ± Standard Deviation	p-value
1000	0.01956±0.002562***	< 0,0001
500	0.01914±0.0008331***	< 0,0001
250	0.03076±0.006123***	< 0,0001
125	0.2848±0.1351***	0,0004
62.5	0.3670±0.08920	0,2151
31.25	0.4034±0.06874	0,8578
15.625	0.4416±0.06340	0,9999
Control (0)	0.4386±0.06989	-

Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001.

MDA Levels

BEAS-2B cells containing 40 µM H_2O_2 and 40 µM H_2O_2 + 15,625 µg/ml *M. communis* L.'s essential oil were incubated for 24 hours and MDA levels in the cells were evaluated. When the control group

(non-H_2O_2) was compared with the 40 µM H_2O_2 group there was a significant increase in MDA level at 40 µM H_2O_2 dose (p=0.0003), whereas there was no significant difference in MDA level between the control and the 40µM H_2O_2+15,625 µg/ml *M. communis* L. dose (p=0,2717) (Figure 2) (Table 4). The oxidative stress group treated with 40 µM H_2O_2 and the treatment group containing essential oil of *M. communis* L. were compared in terms of MDA levels. A significant decrease in MDA levels was detected in the treatment group (p = 0.0009), (Table 4).

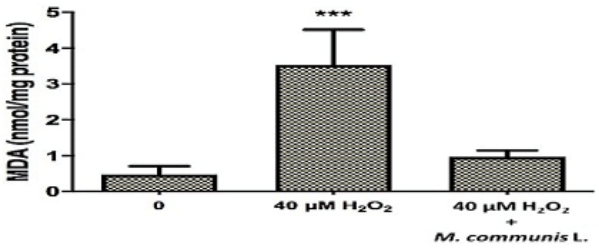

Figure 2. Comparison of MDA in the group of oxidative stress (40µM H_2O_2) and the group with *M. communis* L. (40µM H_2O_2+15,625 µg/ml *M. communis* L) with the control (Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001).

Gene Expression

In the 24th hour of incubation in BEAS-2B cells, the control group and the cell group containing 40 µM H_2O_2 were compared in terms of gene expression levels. In the group exposed to oxidative stress, expression levels of the three genes were found to be significantly increased [Caspase 3; 2.6 fold (p = 0.001), Caspase 8; 2.19 fold (p = 0.017), p21; 10.9 fold (p = 0.0003)], while no significant change was found in the expression level of the Caspase 9 (p = 0.86).

Table 4. MDA levels in BEAS-2B cells according to the groups

Groups	Mean ± Standard Deviation	p-value (0µM compared)	p-value (40µM compared)
0 µM (Control)	0.4638±0.2433	-	0,005
40µM H_2O_2	3.530±0.9798	0,0005	-
40µM H_2O_2 + 15,625µg/ml *M. communis* L.	0.9675±0.1825	0,4270	0,0009

Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001

In the cell group with essential oil of *M. communis* L., the expression level of the Caspase 3 gene was not statistically different to control group (p=0.3). However, there was a significant decrease in this group compared to the group exposed to oxidative stress (p=0.00071). The expression level of the Caspase 8 gene decreased slightly in the group treated with the essential oil of *M. communis* L. compared to the group exposed to oxidative stress (2.07 fold). However, this decrease was not statistically significant (p = 0.93). The expression level of p21 gene was increased by 12.71 fold in the group treated with essential oil of *M. communis* L. (p=0.0003) compared to the control group, and the expression levels in the group in which oxidative stress was induced were found to be similar (p=0.45). No statistically significant difference was found in the expression levels of the Caspase 9 gene in all three groups (Figure 3).

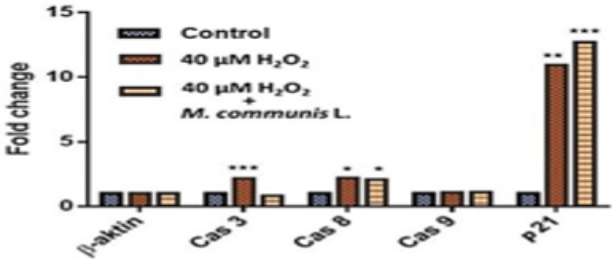

Figure 3. Expression levels of Caspase 3, Caspase 8, Caspase 9 and p21 genes in different groups (Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001)

DISCUSSION

In our study, oxidative stress was induced by exposure to hydrogen peroxide on BEAS-2B bronchial epithelial cells originating from human lung tissue, and the effects of *M. communis* L.'s essential oil on apoptotic pathway and malondialdehyde levels were investigated experimentally. The MTT method was performed to determine the non-cytotoxic concentrations of *M. communis* L.'s essential oil. Subsequently, oxidative stress status and Caspase 3, 8, 9, and p21 gene expressions involved in the apoptotic pathway were evaluated together. This study is valuable in that it demonstrates the beneficial effects of *M. communis* L.'s essential oil on lung cells at the cell level, both on oxidative damage and on apoptosis. As a result of this study, it is an important study that can contribute to the effectiveness of *M. communis* L.'s essential oil in medical applications.

Today, *M. communis* L.'s essential oil, which is rich

in bioactive components, is used in the treatment of various diseases among the population [11]. Kumar et al. reported that different extracts and compounds obtained from the leaves of *M. communis* L. and other parts of the plant have antioxidant activity [12]. To date, although in-vitro studies have been conducted using different forms of *M. communis* L.'s essential oil, studies in this area are very limited [13]. Recently, because of the carcinogenic effects of synthetic antioxidants, their use for human applications has been limited, so the interest of modern medicine in natural antioxidants is increasing [14].

M. communis L.'s essential oil is among the important plants for use in medical applications, as it is a natural source of antioxidants due to the activity of secondary metabolites such as phenylpropanoids and essential oils [15]. Gardeli et al. reported that the antioxidant activities of phenolic compounds are due to redox properties that enable them to act as reducing agents, hydrogen donors, and single oxygen reductants [5]. In our study, unlike these studies, the effects of *M. communis* L.'s essential oil on living cells were investigated not only with oxidative stress but also with apoptotic gene expression.

MTT test was performed to determine the non-cytotoxic doses of essential oil of *M. communis* L. The cells were incubated with different concentrations of the essential oil of *M. communis* L. (1000-500-250-125-62.5-31.25-15.625 µg/ml) for 24 hours. The MTT test showed that the dose of 15,625 µg/ml *M. communis* L.'s essential oil was not cytotoxic dose.

In studies, the scavenging ability of the hydroxyl radical is accepted as a common way to evaluate the potential of antioxidants. It is also believed that the formation of the hydroxyl radical can be achieved by clearing the free metal ions by chelating or converting H_2O_2 to other harmless compounds [16]. In our study, to demonstrate this effect, BEAS-2B cells were treated with different concentrations of H_2O_2 (0, 10, 20, 30, 40, 50 and 75, and 100 µM) cells for 24 hours to determine the appropriate dose and exposure time to form an oxidative stress model. At the end of incubation, cell viability analysis was performed by MTT method (Figure 1.A, Table 2). At the end of 24 hour incubation of H_2O_2 in BEAS-2B cells, no significant difference was observed at a dose of 30 µM compared with the control group. However, it was found that concentrations of 40, 50 and 75, 100 µM of H_2O_2 significantly reduced cell viability. This cytotoxic effect was detected at the strongest level at a dose of 100 µM. The first dose (40 µM dose of H_2O_2) in which cell viability was suppressed to create oxidative stress was chosen as the application dose.

Oxidative stress, which is defined as the imbalance between the levels of various oxidant molecules and antioxidants, causes biochemical changes in many organisms and thus serious diseases [17]. In particular, Park et al. [18] and Barrera et al. [19] reported that oxidative stress caused cytotoxic and genotoxic effects, causing damage to basic biomolecules such as lipids, proteins and DNA, and mediating irreversible damage at the cell level. Consequently, DNA damage resulting from the attack of reactive oxygen species is considered to be the main cause of mutagenesis and carcinogenesis [20]. Romani et al. [21] evaluated the effect of *M. communis* L. extracts on antioxidant activity in their study. In a different study, Ines et al. Reported that some compounds isolated from *M. Communis* L.'s leaf had antioxidant activity [22]. Antioxidant activity was determined in the K562 cell line with its ability to inhibit lipid peroxidation induced by hydrogen peroxide.

TBA test was used to measure MDA, which formed after lipid hydroperoxide decomposition, forming a pink chromophore with thiobarbituric acid. Turhan et al. observed lipid oxidation by determining the peroxide value, thiobarbituric acid reactive substance and oxidative compliance score [23]. In another study conducted in 2015, myrtle and rosemary extracts were shown to be very effective in slowing lipid oxidation, and in this study *M. communis* L. was shown to decrease the MDA value [24]. In our study, similar to these studies, the MDA level was measured to evaluate the effect of *M. communis* L.'s oil on living cells. BEAS-2B cells were incubated with 40 µM H_2O_2 and 40 µM H_2O_2+15.625 µg/ml *M. communis* L.'s essential oil for 24 hours and MDA levels in the cell were measured. Compared to the control group, a significant increase in MDA level was

observed at 40 μM H_2O_2 dose. However, in the group containing 40 mM H_2O_2+15.625 μg/ml *M. communis* L.'s essential oil, MDA levels were similar to the control group. (Figure 2, Table 4). *M. communis* L.'s oil has been able to reduce the oxidative stress caused by H_2O_2 in the cells, indicated by the increase in MDA, to the levels detected in the control cells.

In the study of Bajpai et al., *M. communis* extracts were reported to be a good scavenger of reactive oxygen species [25]. Sahreen et al. showed that hydrogen peroxide reacted with the main cell components, involved in lipid peroxidation, and also caused DNA damage [26]. In a study by Miguel et al., the capacity of H_2O_2 to inhibit the oxidative effect was reported to be directly proportional to the concentration of *M. communis* extracts. This observed H_2O_2 scavenging activity can be attributed to the presence of phenolic compounds that can readily transfer electrons to hydroxyl radicals [27]. Kumar et al. showed that myrtle extracts inhibited lipid peroxidation and thus decreased MDA levels [12].

In our study, the dose selected for H_2O_2 was chosen as the first dose (40 μM) to suppress cell viability in order to induce oxidative stress. In fact, the final non-cytotoxic dose, 30 μM H_2O_2, did not produce a significant increase in MDA levels in cells. However, H_2O_2 at a concentration of 40 μM caused an increase in MDA levels in the cells, and MTT test revealed the parallelism between MDA. In the cell group in which *M. communis* L.'s essential oil was used, MDA values were found to be similar to those of the control group. This shows the antioxidant activity of *M. communis* L.'s essential oil. These results are consistent with the studies of Kumar et al. [12] and Gonçalves [28].

In our study, it was found that the expression of Caspase 3, 8 and p21 genes were significantly increased in BEAS-2B cells at the 24th hour when the control group and the cell group exposed to oxidative stress (40μM H_2O_2) were compared (Figure 3). An increase in p21 expression, a Cdk inhibitor, suggested that the cell stopped the cell cycle in order to decide on repair or apoptosis. There was no significant change in the expression level of the Caspase 9 gene. This shows us that 40 μM H_2O_2 triggers apoptosis in the cell via the extrinsic pathway. Thus, we concluded that this dose, which we found high levels of MDA in the cell, caused apoptosis due to irreparable oxidative damage in the cells.

When the *M. communis* L. group (40μM H_2O_2+15,625μg/ml *M. communis* L.) was compared to the group exposing to oxidative stress (40μM H_2O_2), Caspase 3 expression was reduced in *M. communis* L. treated cells. Although we observed a small decrease in caspase 8 gene expression, this decrease was not statistically significant. However, Caspase 3 gene, which is responsible for the DNA fragmentation stage which is the last step of caspase cascade, decreased below the control levels in the last stage of apoptosis. It showed that *M. communis* L. was highly effective in preventing apoptosis due to oxidative damage.

Fernald and Kurokawa stated that besides targeting apoptosis stimulation, developing a therapeutic strategy to simultaneously reduce oxidative stress in the environment would be a very important approach [29]. The study of Tretiakova et al. in cancer cells is important in elucidating the activation of apoptosis via intrinsic and extrinsic pathways via *M. communis* L.'s essential oil [30]. It has been shown that *M. communis* L. in particular activates apoptosis by caspase 3, 8 and 9 in cancer cells. In our study, unlike these studies, the cell line studied was not a cancer cell line. In cancer therapies, directing apoptosis-resistant cancer cells to apoptosis is an important treatment strategy. In healthy cells, regression of apoptosis due to oxidative damage is extremely important in terms of cell survival.

Limitations: Our study has some budgetary limitations, such as the fact that apoptotic cells could not be demonstrated by more advanced methods such as TUNEL, and gene expression products could not be demonstrated at the protein level. We believe that by using these methods, the antioxidant activity of *M. communis* L.'s essential oil can be better understood by showing apoptotic cells and gene product proteins.

Conclusion: We believe that the essential oil of *M. communis* L. will provide medical benefit because it reduces oxidative stress induced by hydrogen peroxide and simultaneously suppresses apoptosis from oxidative stress. In our study, it

was shown that the essential oil of *M. communis* L. has a strong reducing effect on oxidative stress at a dose of 15.625 µg/ml based on MDA levels. Furthermore, the inhibitory activity on the mechanism of apoptosis induced by oxidative stress at the same dose was determined. As a result, *M. communis* L.'s oil showed a medically strong therapeutic effect both by reducing oxidative stress and by inhibiting oxidative stress induced apoptosis.

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Effects of anxiety sensitivity on nicotine dependence and smoking cessation success

Anksiyete Sensitivitesinin Nikotin Bağımlılığı ve Sigara Bırakma Başarısına Etkileri

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ABSTRACT

Aim: In this study, we aimed to investigate the relationship between the anxiety sensitivity levels and nicotine dependence and smoking cessation outcomes in patients referred to the Smoking Cessation Policlinics.
Methods: This retrospective study included 286 patients referred to a smoking cessation policlinic between January 2017 and July 2017. Socio-demographic characteristics, Fagerström Test for Nicotine Dependence (FTDN) scores, depression scores measured by the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and Anxiety Sensitivity Index-3 (ASI) scale scores were retrospectively retrieved from patient medical files. All patients were contacted and the instant smoking status of the patients was recorded.
Results: Of the participants, 19.5% (n=56) (including those who did not come to follow-up) had quit smoking and were abstinent at least six months after the quitting date. The mean scores of anxiety sensitivity were significantly higher in moderate/high nicotine dependent patients than in mild nicotine dependent patients (p=0.001 and p<0.001, respectively). The mean scores of anxiety sensitivity and all its subscales were significantly higher in current smokers than ex-smokers (p<0.001 for each).
Conclusion: It has been determined that anxiety sensitivity may be a severe barrier to smoking cessation success. Therewithal, anxiety sensitivity is significantly associated with high nicotine dependence. It is essential to evaluate the anxiety sensitivity, anxiety, and depression levels from the first days of patients who are planning to stop smoking. High anxiety sensitivity smokers should be carefully monitored, and treatments should be applied to reduce their anxiety sensitivities to increase quit rates.

Keywords: anxiety, anxiety sensitivity, smoking cessation, nicotine dependence, depression

ÖZ

Amaç: Bu çalışmada, Sigara Bırakma Polikliniğine başvuran hastalarda anksiyete duyarlılık düzeyleri ile nikotin bağımlılığı ile sigara bırakma sonuçları arasındaki ilişkiyi araştırmayı amaçladık.
Yöntem: Bu retrospektif çalışma Ocak 2017-Temmuz 2017 tarihleri arasında sigara bırakma polikliniğine başvuran 286 hastayı içermektedir. Sosyo-demografik özellikler, sigara içme durumu, Nikotin Bağımlılığı için Fagerström Testi (FTDN) skorları, Beck Depresyon Envanteri (BDI) ile ölçülen depresyon skorları ve Beck Anksiyete Envanteri (BAI) ve Anksiyete Duyarlılığı İndeksi-3 (ASI) ölçeklerinin skorları hasta tıbbi dosyalarından geriye dönük olarak alındı. Tüm hastalar ile irtibata geçildi ve hastaların anlık sigara içme durumu kaydedildi.
Bulgular: Katılımcıların % 19,5'i (n = 56) (izlemeye gelmeyenler dahil) sigarayı bırakmış ve sigarayı bırakma tarihinden en az altı ay sonra hala içmemektedirler. Ortalama anksiyete duyarlılığı skorları orta / yüksek nikotin bağımlı hastalarda hafif nikotin bağımlı hastalardan anlamlı derecede yüksekti (sırasıyla p = 0.001 ve p <0.001). Anksiyete duyarlılığı ve tüm alt ölçeklerinin ortalama puanları, mevcut sigara içicilerin sigara içenlere göre anlamlı derecede yüksekti (her biri için p <0.001).
Sonuç: Anksiyete duyarlılığının sigara bırakma başarısında ciddi bir engel olabileceği belirlenmiştir. Bununla birlikte, kaygı duyarlılığı, yüksek nikotin bağımlılığı ile anlamlı şekilde ilişkilidir. Sigarayı bırakmayı planlayan hastaların ilk günlerinden kaygı duyarlılığı, kaygı ve depresyon düzeylerini değerlendirmek önemlidir. Yüksek kaygı duyarlılığına sahip sigara içicileri dikkatle izlenmeli ve bırakma oranlarını artırmak için kaygı hassasiyetlerini azaltmak için tedaviler uygulanmalıdır.

Anahtar Sözcükler: anksiyete, anksiyete duyarlılığı, sigara bırakma, nikotin bağımlılığı, depresyon

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INTRODUCTION

Anxiety sensitivity (AS) has been described as an extreme fear against anxiety-related sensations and statements, that are believed to have harmful physiological and social consequences [1]. Although the recent studies on anxiety sensitivity are aimed at associating the relationship between anxiety and mental disorders, there is a growing consensus that it plays a role in the problems of substance abuse [1,2]. An important one of these substances is cigarettes, which are widely used in society.

It has been shown in smokers that negative mood and mood disorders increase the chance of failure in smoking cessation [2]. For this reason, a better understanding of depressive and anxiety symptoms will benefit clinicians in terms of their clinical goals of improving smoking cessation outcomes. Understanding depressive and anxiety symptoms means identifying the current transdiagnostic factors rather than to focus on symptoms [2,3]. Anxiety symptoms (AS) is precisely such a transdiagnostic factor, that plays a crucial role in the development of these symptoms and increases the risk of developing a disease from the same symptoms. Sensitivity to anxious situations, in other words AS, has been recognized as an individual difference [1,4].

According to the growing scientific research, reducing the adverse effects of high AS is one of the most important reasons for smoking. Smoking is subjectively reducing the anxiety in high anxiety sensitivity smokers when compared with low anxiety sensitivity smokers. In addition, relative to those with lower anxiety sensitivity, smokers with high anxiety sensitivity, report perceiving the prospect of quitting as more difficult and experience more intense nicotine withdrawal during early phases of quitting. Furthermore, anxiety sensitivity explains the relationship between emotional disorders and nicotine dependence, barriers to cessation, and severity of symptoms while quitting [5]. Importantly, anxiety sensitivity is associated with an increased rate of smoking lapse (any smoking behavior) during the early phases of quitting in terms of smoking cessation. Furthermore, these observed anxiety sensitivity and smoking relations are not better explained by the amount of smoking, nicotine dependence, sex, other concurrent substance use (such as alcohol and cannabis), panic attack history, or trait-like negative mood propensity [4].

It will be an innovative approach to conduct these studies in our country and in the smoking cessation outpatient clinics in order to guide physicians when applying smoking cessation therapies, to facilitate treatment, and to determine the sensitivity of the patient's follow-up order to determine the relationship between smoking and cessation. In this study, we aimed to investigate the relationship between the beginning anxiety sensitivity levels and nicotine dependence of patients and smoking cessation outcomes in patients referred to the Smoking Cessation Policlinics.

MATERIALS AND METHODS

This retrospective descriptive study was carried out on patients who were referred to the Düzce University, Department of Family Medicine, Smoking Cessation Policlinic between January and July 2017. 472 patients applied to the Smoking Cessation Polyclinics during that period. At the Smoking Cessation Policlinic, if a patient has a psychiatric illness or psychiatric treatment, we routinely referred this patient to psychiatry. Therefore in this study, patients who were found to be in this situation (32 patients) were excluded from the study. 440 patients were identified and attempts were made to reach them on their registered phones. Of these, some 402 patients were in fact reached and the smoking cessation status of the 286 patients who agreed to participate in the study, were determined by telephone. Patients who had not smoked for at least six months were considered to have quit smoking. The recorded sociodemographic characteristics included age, sex, educational status, occupation, marital status, the age of first smoking experience and smoking initiation, as well as smoking cessation information. The treatments given to the patients were not included in the study. Socio-demographic characteristics, the Fagerström Test for Nicotine Dependence (FTDN) scores, the depression scores measured by the Beck Depression Inventory (BDI), the Beck Anxiety Inventory (BAI) and the Anxiety Sensitivity Index-3 (ASI) scale scores were retrospectively

retrieved from patient medical files. The continuity of smoking cessation status was queried and noted by reaching the patients 6 months after their application to the Smoking Cessation Policlinic.

Ethical approval: All procedures performed in studies involving human participants were done so in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. The study was approved by the local ethics committee at Düzce University Medical Faculty (IRB number: 2017-125, date: 09.10.2017). Informed consent was obtained from all individual participants included in the study.

Study Instruments

The Anxiety Sensitivity Index-3 (ASI-3)

The anxiety sensitivity index was first developed in 1986 by Reiss et al., who also defined “anxiety sensitivity” [4]. This index is a 16-items measure, which utilizes a 5-point Likert-type scale. The instrument is used to assess the degree to which participants are concerned about the possible negative consequences of anxiety symptoms. A score ranging from 0 to 4 can be taken from each item. The scale is subjected to discrete physical, cognitive, and social calculations. Taylor and colleagues reviewed the scale in 2007 and the final state of this scale was named as “Anxiety Sensitivity Index-3”, consisting of 18 items, having three subscales and a score ranging from 0 to 72. The validity and reliability study in Turkish was carried out in 2008 [6].

Fagerström Test for Nicotine Dependence (FTND)

FTND, also known as "Fagerström Tolerance Test", is the most common test designed to assess grading tobacco dependence. FTND consists of six questions. It was developed by Fagerström in 1991 then, reassessed and modified by Heatherton and Kozlowski [7]. The reliability and validity study of FTND was conducted in Turkey and found to be applicable in Smoking Cessation Clinics. Responses to the questionnaire are classified as low (FTCD score ≤3), medium (4–6) and high (≥7).

Beck Depression Inventory (BDI)

The BDI consists of 21 self-rated questions, each answer being scored on a scale of 0–3 giving a score ranging from 0 to 63. The scores are interpreted as; 1–10 points - no depression, 11–16 points - mild mood disturbance, 17–20 points - borderline clinical depression, 21–30 points - moderate depression, 31–40 points - severe depression, and over 40 points - extreme depression. BDI was designed by Beck in 1961 to measure the risk of depression, the severity of depression, depressive symptoms, and depression levels in adults [8]. A study performed by Aktürk et al. stated that a short version of this scale could be used for depression screening in family practice [9].

Beck Anxiety Inventory (BAI)

The BAI scale was developed by Beck et al. in 1988 in response to the need for a scale that was able to distinguish anxiety from depression. It is designed to measure experienced severity of anxiety symptoms. The Beck Anxiety Inventory consists of 21 items and is scored from 0 to 3, based on a Likert scale. The validity and reliability study for a Turkish version of this inventory was performed by Ulusoy al. [10] in 1998. Scores are considered to indicate the following: 0–7, minimal anxiety; 8–15, mild anxiety; 16–25, moderate anxiety; and 26–63, severe anxiety.

Statistical Analysis

The following tests were used for the statistical analyzes of our study: the distribution of continuous variables was examined by the Shapiro-Wilk test, the independent Samples t-test - or Mann-Whitney U test - was used to compare two independent groups, and the One-Way ANOVA or Kruskal-Wallis tests were used to compare more than two independent groups. The Pearson Chi-Square or Fisher's exact tests were used to analyze categorical data. The Pearson or Spearman correlation analysis was used, depending on the distribution of variables, in examining correlations between continuous variables. Statistical analyzes were performed with the Statistical Packages for the Social Sciences (SPSS) v.22 and the level of significance was set at 0.05.

RESULTS

The sample included 194 (67.8%) male and 92 (32.2%) female participants. Fifty-six (19.5%) of all participants reported that they had quit smoking while 230 (80.4%) were current smokers. The mean age of ex-smokers was 38.07 ± 14.75 years, while the mean age of current smokers was 39.40 ± 13.09 years. There was no statistically significant difference between the mean ages of ex and current smokers (t=-0.666; p=0.506). 19.5% (n=38) of the male participants and 19.5% (n=18) of females ad quit smoking; smoking cessation rates were not statistically different between the two genders (Chi-Square=0.000; p=0.996). Additionally, 69.6% (n=39) of the quitters were married while 73% (n=168) of current smokers were married and the smoking cessation rate-increase in married participants was statistically insignificant (Chi-Square=0.260; p=0.610).

Although none of the farmers had quit smoking, 14.5% (n=8) of the unemployed participants, 27% (n=19) of the workers, 20% (n=8) of the civil servants, 29% (n=9) of the retirees, 11.1% (n=2) of the self-employed, 25.8% (n=8) of the students, and 18.2% of the private sector employees (n=2) had quit. The difference between occupations concerning smoking cessation rates was not statistically significant (Chi-Square=6.260; p=0.510). The median smoking rate of quitters was 15 (1-76) pack/year, while this rate for the current smokers was 18 (1-179) packet/year; this difference was not statistically significant (Z=-1.535; p=0.125). The mean age of the first smoking experience of the quitters was 14.79±3.66 years, whereas this figure was 14.78±4.99 years for the current smokers. There was no statistical difference in terms of age of the first smoking experience (t=0.011; p=0.992). The mean age of the quitters to start smoking was calculated as 17.02±3.96 years, while the same for current smokers was 16.75±4.91 years and there was no statistical difference in terms of smoking initiation ages (t=0.376; p=0.707).

The median values of total depression scores were significantly lower in ex-smokers than current smokers (Z=-2.763; p=0.006). The median values of total anxiety scores were significantly lower in ex-smokers compared to current smokers (Z=-5.033; p<0.001). The median values of total, physical, social, and cognitive ASI-3 scores were also significantly lower in quitters than current smokers (Z and p -6.830, <0.001; -6.225, <0.001; -6.463, <0.001; and -5.983, <0.001, respectively) (Table 1). Additionally, smoking cessation rates were higher in participants who had mild FTND scores than those with high or moderate FTND scores (Table 2).

Table 1. Factors affecting successful smoking cessation

	Ex-smokers (n=56) Median (range)	Current smokers (n=230) Median (range)	p
Beck depression score	10 (0-43)	15 (0-55)	0.006
Total ASI-3 score	13 (0-47)	30 (1-72)	<0.001
Physical ASI-3 score	8 (0-24)	15 (0-28)	<0.001
Social ASI-3 score	3 (0-16)	8 (0-28)	<0.001
Cognitive ASI-3 score	4 (0-16)	8 (0-24)	<0.001
Beck anxiety score	12 (0-32)	25 (0-52)	<0.001

Table 2. The relation of FTND scores to smoking cessation

	Smoking Status			
	Ex-smoker		Current smoker	
FTND score	n	%	n	%
Low (<4)	22	38.6	35	61.4
Medium (4-6)	15	12.2	108	87.8
High (>6)	19	38.6	87	82.1

Chi-Square=17.535, p<0.001

Nicotine dependence levels were highly related to the Beck Depression scores and the Beck Anxiety scores between smoking group and ex-smokers (p=0.002 and p<0.001, respectively) (Table 3).

The relationship between nicotine dependence levels and ADI-3 total, physical, cognitive, and social scores between smoking group and ex-smokers was also statistically significant (p=0.001, p<0.001, p= 0,02, p=0,007 respectively) (Table 3).

Anxiety had a weak correlation with social anxiety sensitivity whereas it had a moderate correlation with physical and cognitive anxiety sensitivity. Depression and anxiety levels were significantly correlated with nicotine dependence (Table 4).

DISCUSSION

In this study, smoking cessation rate was determined to be19.5%, based on the patients

Table 3. The relationship between FTND groups, ası-3 Scores and Beck Depression Inventory scores, and Beck Anxiety Inventory scores

	Mild (≤3) (n=57) Median (range)		Moderate (4-6) (n=123) Median (range)		High (≥7) (n=106) Median (range)		Kruskal-Wallis Z, p
	Ex-smoker (n=22)	Current smoker (n=35)	Ex-smoker (n=15)	Current smoker (n=108)	Ex-smoker (n=19)	Current smoker (n=87)	
Total ASI-3 score	17 (2-45)	25 (2-52)	29 (0-65)	30 (2-72)	26 (0-49)	28 (2-59)	14.254, 0.001
Physical ASI-3 score	7 (0-19)	12 (0-21)	11 (0-24)	16 (0-28)	13 (0-24)	16 (0-26)	15.964,<0.001
Social ASI-3 score	5 (0-20)	7 (0-21)	8 (0-25)	9 (0-28)	6 (0-20)	8 (0-21)	7.798,0.020
Cognitive ASI-3 score	5 (0-18)	8 (0-19)	6 (0-22)	10 (0-24)	6 (0-19)	8 (0-21)	9.890, 0.007
Beck Depression Inventory scores	8 (1-30)	12 (1-35)	10 (0-44)	15 (1-51)	17 (0-55)	21 (1-55)	12.481, 0.002
Beck Anxiety Inventory scores	10 (0-40)	15(1-43)	18 (0-47)	25 (1-55)	19 (1-48)	25(1-52)	15.889,<0.001

Table 4. Correlations of ASI-3 data

		FTND	Beck Depression Inventory	Beck Anxiety Inventory	Physical ASI-3	Cognitive ASI-3	Social ASI-3	Total ASI-3
FTND	r		0.234	0.204	0.115	0.081	0.045	0.104
	p		<0.001	0.001	0.053	0.174	0.450	0.080
Beck Depression Inventory	r	0.234		0.291	0.223	0.262	0.250	0.269
	p	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001
Beck Anxiety Inventory	r	0.204	0.291		0.636	0.552	0.385	0.615
		0.001	<0.001		<0.001	<0.001	<0.001	<0.001

who quit smoking and remained abstinent for six months. Many different ratios, ranging from 29.1% to 45.5%, were reported from other smoking cessation clinics in Turkey [10,11]. Therewithal, another study [18] found a smoking cessation rate of 44.2% for the first year and 48% for the first six months. The reason of these high rates may be related with the calculation methods, as some of the calculations were done based on the patients who continued to follow-up instead of including all patients referred to the clinic. Certainly, the frequency of motivational interviews and patient follow-ups through phone calls may also have contributed to the quit rates. A study performed in Ankara Ataturk Training and Research Hospital, which investigated the reasons of smoking continuation demonstrated a cessation rate of 10.9% [12]. A wide range of smoking cessation rates can be seen when we look at the previous studies in general; more clear and standardized smoking cessation rates should be established by conducting joint and more comprehensive studies using standard measures.

Smoking cessation rates were lower in patients who had moderate and high depression. Some studies surveying the reasons of keeping smoking in patients who had depressive symptoms or high levels of depression, revealed that although smokers with generally high levels of depressive symptoms had some smoking cessation motivation or self-efficacy, they were more motivated to keep smoking [13]. Numerous psychiatric studies are available in the literature pointing to the effects of depression as a barrier to smoking cessation [14,15]. Depression and anxiety sensitivity were positively correlated in our study.

Nicotine dependence correlated positively with anxiety levels. Anxiety and nicotine dependence are paradoxical occurrences that can trigger each other in adolescence [16]. There are two basic hypotheses explaining nicotine dependence and increased risk of some anxiety disorders in early adolescence and early adulthood. The first hypothesis claims that a person becomes dependent on actions involving tobacco use due to its facilitation in coping with anxiety, its sedative effects, its social interaction, and peer pressure in anxious individuals. The other hypothesis says

that smoking (on account of nicotine dependence) increases the incidence, symptoms, and risk of anxiety disorders [17]. A group of researchers pointed out that smoking could lead to anxiety disorders, but anxiety disorders do not increase smoking risk [17]. Our study does not shed light on this topic because it is based on the results, not the reasons. To further elucidate this issue, a wide range of studies targeting young people are needed. However, based on anecdotal reports of smokers and empirical work consistent with these reports, it is possible to emphasize that tobacco and nicotine may have anxiolytic effects.

Anxiety and depression levels were positively correlated with anxiety sensitivity. As nicotine dependence increased, anxiety sensitivity scores increased too. A prospective study on 119 patients indicated that increased anxiety and AS caused an increase in nicotine withdrawal symptoms during the first week of cessation. The same study also claimed that smoking cessation levels had decreased for the first month following an increase in the AS levels [18]. The barriers to motivation of smoking cessation were studied and the possible barriers were defined as panic attack history, daily smoking amount, and high levels of AS. Johnson and his colleagues conducted a study with 123 participants and had two outcomes. One was the significant relationship between AS and anxiety. The other was the association between the increased nicotine withdrawal symptoms and increased anxiety and AS [19]. The intensity of nicotine withdrawal symptoms and the barriers to quit smoking in the early phase of smoking cessation programs bring the following question: "Can we increase the smoking cessation success if we reduce the anxiety sensitivity?" A study reported that smoking cessation group therapy was applied to six participants who were admitted to the AS reduction program and it was reported that reducing AS significantly improved smoking cessation [20]. Similarly, another study conducted among the individuals who managed to quit smoking reported that reduction of AS and use of nicotine replacement therapy caused rapid retraction of nicotine withdrawal symptoms after smoking cessation [21]. A scientific study targeting AS, anxiety, and smoking cessation and including patients who were assessed empirically for six months proved that AS decreased with the treatment of anxiety; this decline was maintained for six months and there was limited evidence that this increased the motivation to quit smoking [20,21].

Our results pointed out that there is a relationship between AS, nicotine dependence and smoking cessation. Additionally, we can state that anxiety sensitivity can be a significant factor in the effort to quit smoking. Based on the current evidence, we can conclude that AS and smoking increase anxiety disorders, depression, and nicotine dependence.

Smoking can assume important regulatory functions in individuals with high levels of anxiety sensitivity. These individuals are particularly smoking to help reduce their anxiety. High anxiety-sensitive smokers can learn to apply smoking to manage their feelings in the short term, when strategies to tackle their problems become inadequate. However, being a smoker may gradually increase nicotine withdrawal symptoms, health impairment, and loss of balance in internal dynamics for various reasons [22,23]. These dissuasive elements also teach the person their concern about being harmed. This concern may provide motivation for smoking cessation in high anxiety-sensitive smokers; however, these people feel the nicotine withdrawal symptoms more intensely as well as increasing depressive and anxious symptoms and therefore, may have a higher risk of not being able to stop smoking. In addition, believing in dissuasive elements of smoking cessation and continuing to smoke in the short term can, paradoxically, create a risk for anxiety disorders in the long term.

Limitations of the Study: Several limitations of the present investigation should be considered. First, it was not possible to obtain data for depression and anxiety diagnoses; evaluations were done with the Beck Depression Inventory and Beck Anxiety Inventory scales. Second, we did not measure the level of nicotine withdrawal or the intensity of withdrawal symptoms after smoking cessation. Third, it may be more effective to examine the withdrawal symptoms throughout the duration of the cessation attempt: smoking cessation outcomes (lapse and relapse) were ignored in the present study. Fourth, since our

work was limited to patients who applied to our clinic, it is not possible to extend our results to the general population. The relationship between anxiety sensitivity and smoking cessation can be further clarified by applying an algorithm that investigates the causes of barriers and motivation losses of patients. And, of course, we could not evaluate the effects of anxiety and depression on smoking cessation, since we did not measure and compare the anxiety and depression scores.

As a result, the relationships between tobacco use, nicotine dependence, anxiety sensitivity, anxiety disorders and depression are complex and unclear. An interdisciplinary approach is needed to assist patients at risk. The results of our study show that anxiety sensitivity is effective in smoking cessation behavior and sheds light on other reasons that may prevent smoking cessation. In light of our results, new approaches to increase the success of smoking cessation can be established with follow-up and treatment studies taking into account the anxiety sensitivity of individuals, in smoking cessation polyclinics and addictive polyclinics.

CONCLUSION

Our results showed that anxiety sensitivity, especially physical and cognitive anxiety sensitivity, made smoking cessation particularly difficult. Anxiety sensitivity was significantly higher in those with high nicotine dependence. An increase in nicotine dependence reduced the success of smoking cessation. This issue made us consider that success in smoking cessation depends mainly on the anxiety sensitivity and its effects on nicotine dependence.

Consistent with our hypothesis, AS was also closely associated with anxiety and depression. The results of our study showed that an increase in anxiety and depression levels significantly reduce smoking cessation success. Additionally, we can say that AS may also prevent smoking cessation through these pathways when taking the undeniable negative effects of anxiety and depression on smoking cessation success into account.

The associations between tobacco smoking, nicotine dependence, anxiety sensitivity, anxiety disorders, and depression are complex and unclear. An interdisciplinary approach is needed to help patients under these risks. At this point, further and more detailed studies are needed to explore the mutual relationship of etiologic factors. Although the effect of smoking on objective mood is complex, these processes can be conceptualized using cognitive analysis. The results of our study indicate that high anxiety sensitivity may be a barrier to smoking cessation.

In light of these findings, it can be suggested that new approaches should be sought to increase the success of smoking cessation using follow-up and treatment studies considering anxiety sensitivities of the patients.

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The contribution of power Doppler mode of endobronchial ultrasound (EBUS) used in mediastinal and hilar lymphadenopathies in the differentiation of benign and malignant lymph nodes

Mediastinal ve hiler lenfadenopatilerde endobronşiyal ultrason eşliğinde kullanılan power Doppler modunun benign ve malign lenf nodu ayırımına katkısı

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ABSTRACT

Aim: The aim of this study was to investigate the contribution of power Doppler mode used in the differentiation of malignant and benign lymph nodes in patients with mediastinal and hilar lymphadenopathies undergoing diagnostic fine needle aspiration under the guidance of convex probe endoscopic ultrasound (EBUS).

Material and Methods: Medical files of patients who underwent EBUS between March 2018 and February 2019 were retrospectively analyzed. A total of 143 lymph nodes of 85 patients were included in the study. The demographic characteristics of the patients with a definite diagnosis and EBUS radiological and sonographic characteristics of the sampled lymph nodes were recorded. As a result of the evaluation of mediastinal lymph nodes with EBUS, the sensitivity, specificity, negative and positive predictive values were calculated for power Doppler mode in the detection of malignant lymph nodes.

Results: Of a total of 85 patients, 62 (73%) were males and 23 (27%) were females with a mean age of 62±10.4 (range, 37 to 80) years. In the vascular pattern evaluation, 87 (60.8%) lymph nodes were negative and 56 (39.2%) were positive for malignancy. Comparing final diagnoses and vascular pattern analysis results revealed a statistically significant difference between benign and malignant lymph nodes in favor of power Doppler mode under the guidance of EBUS (p <0.01).

Conclusion: Convex probe EBUS can identify the first lymph node to be sampled based on sonographic characteristics of lymph nodes and allow simultaneous imaging and transbronchial fine needle aspiration procedures. Therefore, it may shorten the procedural time and reduce the amount of anesthesia to be used.

Keywords: Mediastinal lymph node; mediastinal staging; power doppler ultrasonography.

ÖZ

Amaç: Bu çalışma ile konveks prob endobronşiyal ultrasonografi (EBUS) eşliğinde tanısal amaçlı ince iğne aspirasyon yapılan olgularda, power Doppler modunun mediastinal ve hiler lenfadenopatilerde malign ve benign ayrımına katkısı araştırıldı.

Gereç ve Yöntemler: Mart 2018 ile Şubat 2019 tarihleri arasında EBUS yapılan olguların tıbbi dosyaları retrospektif olarak incelendi. Toplam 85 olguya ait 143 lenf nodunun örnekleme sonuçları çalışmaya dahil edildi. Kesin tanısı olan olguların demografik özellikleri, örneklenen lenf bezlerinin EBUS'daki radyolojik ve sonografik özellikleri kayıt edildi. Mediastinal lenf nodlarının EBUS ile değerlendirilmesi sonucunda power Doppler modunun malign lenf nodu belirlemedeki duyarlığı, özgüllüğü, negatif ve pozitif prediktif değeri hesaplandı.

Bulgular: Çalışmaya dahil edilen 85 hastanın 62'si (%73) erkek ve 23'ü (%27) kadın olup, yaş ortalaması 62±10,4 (dağılım: 37-80) yıl idi. Vasküler patern değerlendirmesi sonucunda malignite açısından negatif lenf nodu sayısı 87 (%60,8) iken, pozitif olarak değerlendirilen lenf nodu sayısı 56 (%39,2) olarak saptandı. Kesin tanıları ile vasküler patern analizi karşılaştırıldığında, EBUS eşliğinde kullanılan power Doppler modunun benign ve malign lenf nodu ayrımında katkısı istatistiksel olarak anlamlı bulundu (p<0,01).

Sonuç: Konveks prob EBUS, eş zamanlı görüntülemeye ve transbronşiyal ince iğne aspirasyon uygulamalarına olanak sağlamasının yanı sıra, lenf nodlarının sonografik özelliklerini tanımlayarak ilk örneklenecek lenf nodunu tespit edebilir. Bu da, işlem süresinin kısalmasını ve hastanın alacağı anestezi miktarının azalmasını sağlayabilir.

Anahtar Kelimeler: Mediastinal lenf nodu; mediastinal evreleme; power Doppler ultrasonografi.

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INTRODUCTION

Transbronchial needle aspiration under the endobronchial ultrasound guidance (EBUS-TBNA) is a safe, minimally invasive procedure which can be applied under local anesthesia with a high diagnostic rate in the diagnosis and/or grading of lung cancer (1). Materials for histological and cytological evaluation can be obtained from mediastinal and hilar lymph nodes with EBUS-TBNA (2). In addition to lung cancer, the effectiveness of this modality has been shown in the diagnosis of sarcoidosis, tuberculosis, and lymphoma (3, 4).

During the EBUS procedure, the sonographic characteristics of lymph nodes can be evaluated using B mode. The lymph node diameter, shape, borders, echogenicity, central hilar structure, and necrosis can be evaluated using this mode (1). The pathological lymph node can be identified and selected initially, and a sample can be, then, taken. Therefore, EBUS-TBNA is expected to shorten the procedural time (5).

Ultrasonography (USG) is an examination made on the basis of the difference in the amplitude of sound waves. Doppler USG is an imaging modality which uses high-frequency sound waves. When a high frequency sound wave encounters a moving structure such as blood flow within a vessel, the reflected sound wave returns at a different frequency. The returning sound waves can be transformed to an audible signal, which is known as the Doppler effect (6).

There are two types of EBUS device, namely radial and convex probe. Radial probe EBUS (RP-EBUS) using high frequency (20 MHz) can be used to guide transbronchial needle aspiration to determine the depth of bronchial wall invasion in early stage bronchial cancers, to visualize tumor invasion, and to facilitate the diagnosis of lesions with peripheral localization. Convex probe EBUS (CP-EBUS) is used at low frequency (7.5 MHz) for the diagnosis of granulomatous diseases such as sarcoidosis and tuberculosis, for mediastinal grading in lung cancer, and to identify mediastinal masses and diseases (7).

With the effect of angiogenetic factors in neovascularization, a peripheral or mixed bleeding pattern (central and peripheral) occurs in malignant lymph nodes. These flows, which are elevated compared to benign lymph nodes, can be determined using power Doppler mode (8). By measuring the vascularity of lymph nodes in various organs with power Doppler mode, it can be used in the differentiation of metastatic and non-metastatic lymph nodes (9).

In the present study, we aimed to investigate the contribution of power Doppler mode in the differentiation of mediastinal and hilar lymph nodes in patients undergoing CP-EBUS for diagnostic purposes.

MATERIAL and METHODS

Study Population

This retrospective study was conducted at thoracic diseases outpatient clinic of xxx Training and Research Hospital between 1st March, 2018 and 28th February, 2019. Approval for this study was granted by the Clinical Research Ethics Committee of Antalya University Training and Research Hospital (No. 9/13, Date: 14.03.2019). A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Medical files of patients who underwent CP-EBUS procedure for diagnosis and/or grading were retrospectively analyzed. Throughout the study period, 160 lymph nodes were sampled from 96 patients. A total of 17 lymph nodes of 11 patients were excluded from the study due to the following reasons: lost-to-follow-up, having a non-diagnostic cytology diagnosis, or having lymphoma and malignant tumor metastasis other than the lungs based on the clinical and pathology examination results. Finally, a total of 143 lymph node samples from 85 patients were included in the study. Demographic data including age and sex were collected from the patient records, and the mediastinal distribution of the lymph nodes taken for biopsy and the pathological final diagnoses were noted.

In addition, the lymph node dimensions were determined on computed tomography (CT), and the sonographic characteristics of the lymph nodes and vascular pattern distribution from video

recordings of the procedure. According to the pathological final diagnosis, the effectiveness of the power Doppler USG in the differentiation of benign and malignant lesions was evaluated.

Radiological evaluation

Contrast-enhanced thoracic CT was performed in all patients. The EBUS-TBNA procedure was applied to the patients with a short axis of ≥10 mm in mediastinal-hilar lymph nodes on CT.

EBUS-TBNA procedure

All EBUS-TBNA procedures were performed in the operating theatre under conscious sedation (midazolam+propofol), using a Fujinon EBUS device (7.5mhz EB-530US/Sonart SU-1, Tokyo, Japan). Systematic inspection of mediastinal, hilar, and interlobar lymph nodes (2R, 4R, 10R, 11R, 7, 11L, 10L, 4L, 2L, etc.) was performed using EBUS. Nodal sampling from N3 to N2 and then to N1 was performed. (10,11). Identification of mediastinal lymph nodes was made according to the International Association for the Study of Lung Cancer (IASLC) criteria (12). At least two lymph node stations of all cases were sampled and at least three samples were taken for each lymph node.

Lymph node evaluation

The EBUS video images of the patients were retrospectively examined by two experienced thoracic diseases specialists. During the evaluation, the size of the lymph node, shape, borders, echogenicity, central hilar structure, and presence of necrosis were examined. Vascularity of the lymph node from the power Doppler mode recording was graded as defined in the literature (13). Accordingly, the grading was as follows: Grade 0, no blood flow; Grade 1, vascularity toward the center of the lymph node; Grade 2, visualization of two or three blood vessels in the form of a point, rod or long line; Grade 3, visualization of more than four blood vessels in point, rod, or line form (Figure 1). Lymph nodes with a vascular pattern of Grade 0-1 were considered negative and those of Grade 2-3 positive for malignancy.

Pathological evaluation

The part of the cytological material obtained was prepared by the cytopathologist as smear preparations stained with the Diff-Quik for rapid evaluation of each patient. The remaining material was placed in a box containing cell protective solution (10% formaldehyde) for the formation of cell block. As a result of the cytopathological evaluation, the diagnoses of the patients were reported as i) non-diagnostic cytology, ii) benign cytology, and iii) malignant cytology (a-adenocarcinoma [Figure 2], b-squamous cell carcinoma [SCC], c-carcinoma other than small cell, unclassified, d-small cell carcinoma).

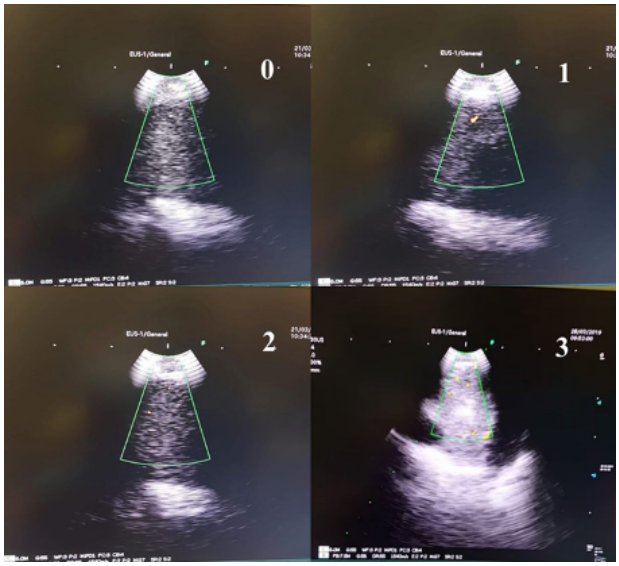

Figure 1. Grading according to vascularity of the lymph node.

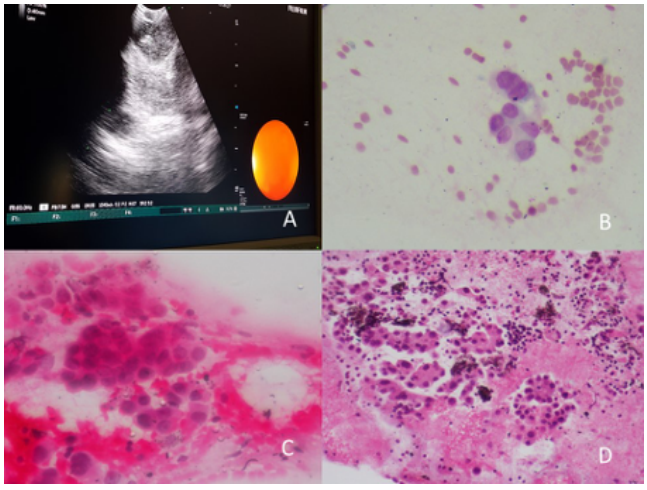

Figure 2. Lung adenocarcinoma: A-Vascular pattern is grade 0. B-C.Tumor cells with nuclear contour irregularity in smear preparations.(Giemsa, x400 and H&E, x400). D-Tumor cells forming adenoid structures in cell block (H&E, x200).

Patient follow-up

Lymph nodes were considered benign in cases where tumoral cells were not detected on EBUS-TBNA and confirmed histopathologically with lymph node dissection, or where no disease progression was observed clinically and radiologically within the past six months of clinical follow-up at least. Lymph nodes were considered malignant based on the visualization of the presence of tumoral cells on EBUS-TBNA or based on histological results with surgical excision.

Statistical Analysis

Statistical analysis was performed using the SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation, median (min-max), or number and percentage. For the comparison of definitive diagnostic rates of the groups with vascular pattern analysis, the chi-square test was used. To analyze the differences in the measurement values according to the groups of definitive diagnoses and to examine the vascular pattern analysis of the lymph node diameter measurements, the t-test was applied. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy were calculated with standard methods. A p value of <0.05 was considered statistically significant.

RESULTS

Of a total of 85 patients, 62 (73%) were males and 23 (27%) were females with a mean age of 62±10.4 (range, 37 to 80) years. Of 143 lymph nodes sampled, 75 (52.4%) were benign and 68 (47.6%) were malignant. The diagnosis of seven of benign lymph nodes was confirmed with mediastinoscopy, while the others were accepted as benign after a six-month clinical and radiological follow-up.

The sampling frequency of the lymph node stations was 3.5% from right upper paratracheal (2R), 0.7% from retrotracheal (3P), 37.8% from right lower paratracheal (4R), 4.2% from left lower paratracheal (4L), 30.8% from subcarinal (7), 8.4% from right hilar (10R), 5.6% from right interlobar (11R), and 9.1% from left interlobar (11L). The mean lymph node diameter was measured as 18.54±7.88 mm on CT and 19.52±7.89 mm sonographically. Lymph node diameters and pathological diagnoses of the sampled lymph node stations are summarized in Table 1.

Table 1. The diameters and pathology diagnoses of sampled lymph node stations

		n	%
Lymph node stations	2R	5	3.5
	3P	1	0.7
	4R	54	37.8
	4L	6	4.2
	7	44	30.8
	10R	12	8.4
	11R	8	5.6
	11L	13	9.1
Lymph node diameter		BT	EBUS
Mean (mm)		18.54±7.88	19.52±7.89
Pathology diagnosis		n	%
Adenocarcinoma		30	21
SCC		12	8.4
NSCLC-unclassified		12	8.4
SCLC		14	9.8
Benign		75	52.4

Data are given in number and percentage, unless otherwise stated. SCC: squamous cell carcinoma; SCLC: small cell lung carcinoma; NSCLC: non-small cell lung carcinoma; CT: computed tomography; EBUS: endobronchial ultrasonography

In the grading of the lymph nodes, 32 (22.4%) were reported as Grade 0, 55 (38.4%) as Grade 1, 44 (30.7%) as Grade 2, and 12 (8.5%) as Grade 3. In the vascular pattern evaluation, 84% of the lymph nodes diagnosed as benign were determined with a negative (Grade 0-1) vascular pattern, while 64.7% of the malignant lymph nodes were determined with a positive vascular pattern (Grade 2-3) (Table 2). The differentiation of the benign and malignant lymph nodes with vascular pattern analysis was found to be statistically significant ($p<0.01$). As a result of the evaluation of the mediastinal lymph nodes with CP-EBUS using the power Doppler mode, the sensitivity, specificity, NPV, PPV, and diagnostic accuracy rates of the identification of malignant lymph nodes were 64.7%, 84.0%, 72.4%, 78.6%, and 74.8%, respectively.

There was a significant correlation between the vascular pattern analysis of the patients and the EBUS diagnosis subgroups. Among the patients with a benign diagnosis, the vascular pattern was Grade 0-1 in 84% and Grade 2 in 16%. In the patients with a malignant diagnosis, subtype analysis revealed Grade 2-3 lung adenocarcinoma

in 50%, Grade 2-3 small-cell lung cancer (SCLC) in 71.4%, and Grade 2-3 SCC in 83.3% of the patients. A total of 75% of lymph nodes were Grade 2-3 histological subtype of unclassified non-small cell lung cancer (NSCLC, unclassified) (chi-square: 64.20, p=0.01, p<0.05) (Table 3).

Table 2. Grading of vascular patterns and definitive diagnoses

		Definitive diagnosis		X^2	p
Vascular pattern		Benign (n=75)	Malignant (n=68)	35.51	0.01
Grade 0-1 (n=87)	n	63	24		
	%	84.0%	35.3%		
Grade 2-3 (n=56)	n	12	44		
		16.0%	64.7%		

Data are given in number and percentage, unless otherwise stated.

Table 3. Correlation analysis results

VP		Tumor subtypes				X^2	p
		LA (n=30)	NSCLC unclassified (n=12)	SCLC (n=14)	SCC (n=12)		
Grade 0-1	n	15	3	4	2	15.29	0.01
	%	50%	25%	28.6%	16.7%		
Grade 2-3	n	15	9	10	10		
	%	50%	75%	71.4%	83.3%		

Data are given in number and percentage, unless otherwise stated. VP: vascular pattern; LA: lung adenocarcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung carcinoma; NSCLC: non-small cell lung carcinoma.

DISCUSSION

In the present study, we evaluated the diagnostic value of power Doppler mode in the differentiation of mediastinal and hilar lymph nodes in patients undergoing CP-EBUS. The results of the study showed that vascular pattern analysis with power Doppler mode was statistically significantly effective in the identification of malignant lymph nodes.

The advent of EBUS at the beginning of the 21st century has dramatically changed the field of thoracic medicine. Later on, EBUS-TBNA has rapidly become the mainstay for the staging of lung cancer and for the diagnosis of mediastinal and hilar lymphadenopathies (1). Owing to the properties of power Doppler mode integrated into the EBUS devices, the vascularity of lymph nodes can be evaluated during the procedure.

The USG Doppler mode findings of malignant tumors are different from those of benign tumors (14) and it has been shown that these findings can be used to differentiate metastatic and non-metastatic lymph nodes in patients with malignant disease (15). In our study, 143 lymph nodes were sampled and 75 (52.4%) were found to be benign, while 68 (47.6%) were malignant. The most common mediastinal lymph node stations sampled were the right lower paratracheal (37.8%) and subcarinal (30.8%) lymph node stations. In a retrospective study of 1,061 lymph nodes, Fujiwara et al. (1) reported the frequency of sampling from the right lower paratracheal and subcarinal lymph node stations to be 31.5% and 27.1%, respectively. These results are consistent with our findings. More frequent sampling from the right lower paratracheal and subcarinal lymph node stations can be attributed to the ease of sampling from these locations with EBUS and that they are more often involved in benign diseases such as sarcoidosis and tuberculosis.

The lymph nodes in the current study were found to be Grade 0 in 32 (22.4%), Grade 1 in 55 (38.4%), Grade 2 in 44 (30.7%), and Grade 3 in 12 (8.5%) cases. According to the vascular pattern analysis, 84% of 75 lymph nodes diagnosed as benign were negative and 64.7% of 68 lymph nodes diagnosed as malignant were positive (p<0.01). In a study, Nakajima et al. (13) evaluated 173 lymph nodes and defined Grade 0-1 vascular pattern as negative and Grade 2-3 as positive for malignancy. In the CP-EBUS evaluation, sensitivity was found to be 87.7%, specificity to be 69.6%, NPV to be 71.7%, PPV to be 86.5%, and diagnostic accuracy to be 78% for the identification of malignant lymph nodes. In another study by Demirci et al. (16), sensitivity was 83.2%, specificity was 66.7%, NPV was 69.7%, PPV was 82.3%, and diagnostic accuracy was 74% for the identification of malignant lymph nodes. Based on the evaluation of 143 lymph nodes of 85 patients in the current study, we found a sensitivity of 64.7%, a specificity of 84%, a NPV of 72.4%, a PPV of 78.6%, and a diagnostic accuracy of 74.8%, consistent with previous findings. However, relatively low sensitivity rates in our study can be explained by the retrospective nature of the study and subjective analysis of video images which might have increased the assessment bias.

To the best of our knowledge, there is a very limited number of studies in the literature using the vascular pattern analysis with EBUS (13, 16). In the present study, beyond the scope of previous studies, we also examined the relationship between lung cancer tumor subtypes and vascular pattern analysis of lymph nodes diagnosed as malignant. The vascular pattern analysis was negative in 50% of malignant lymph nodes of adenocarcinoma subtype. In the other tumor subtypes, the vascular pattern analysis result was determined as positive in 71.4% of SCLC lymph nodes and in 83.3% of those diagnosed with SCC. Those with Grade 2-3 vascular pattern were considered positive in the current study, and this method was found to be more valuable in cases diagnosed with SCLC and SCC.

Nonetheless, there are some limitations to this study. The main limitations include its retrospective nature, relatively low number of lymph nodes sampled, and probability of subjective bias in the evaluation of the EBUS Doppler mode video images.

CONCLUSION

In conclusion, the vascular pattern of lymph nodes can be accurately measured by EBUS using the Doppler mode in patients with lung malignancies. The sampling of lymph nodes with a greater vascularity pattern during the procedure would not only shorten the duration of procedures, but also reduce the amount of anesthesia to be administered. However, further large-scale, prospective studies are needed to establish a definite conclusion.

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Is diabetes mellitus associated with malnutrition in patients in intensive care unit? Diabetes and malnutrition

Yoğun bakım hastalarında diyabetes mellitus malnütrisyonla ilişkili midir? Diyabet ve malnütrisyon

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ABSTRACT

Aim: Types of diseases and treatment modalities can also lead to the exacerbation of malnutrition. The aim of this study was to investigate nutritional status of patients with diabetes mellitus in the intensive care unit of a tertiary hospital.
Materials and methods: One hundred and ninety-two patients were enrolled and divided into two groups. The study group comprised of 77 patients with type 2 diabetes and the control group comprised of 115 patients without diabetes. The nutritional risk assessment was tested with NRS (Nutritional Risk Screening) 2002, Nutric score, MNA (Mini-Nutritional Assessment) and MUST (Malnutrition Universal Screening Tool).
Results: The groups were comparable according to the NRS 2002 (3.37 ± 1.84 vs. 3.93 ± 1.72, p = 0.075), Nutric score (4.61 ± 1.85 vs. 4.56 ± 1.85, p = 0.869), MNA (8.0 ± 3.1 vs. 7.1 ± 3.2, p = 0.068) and MUST score (1.62 ± 1.46 vs. 1.81 ± 1.59, p = 0.456).
Conclusion: In this study, the risk of malnutrition is comparable in both groups. This result may suggest that malnutrition is also related to co-morbidities in addition to diabetes.

Keywords: Diabetes mellitus, malnutrition, intensive care unit

ÖZ

Amaç: Hastalık türleri ve tedavi yöntemleri yetersiz beslenmenin alevlenmesine yol açabilir. Bu çalışmada, üçüncü basamak bir hastanenin yoğun bakım ünitesinde (YBÜ) diyabetes mellituslu hastaların beslenme durumunun araştırılması amaçlandı.
Yöntem: Yüz doksan iki hasta çalışmaya dahil edildi ve iki gruba ayrıldı. Çalışma grubuna tip 2 diyabetli 77 hasta, kontrol grubuna ise diyabeti olmayan 115 hasta alındı. Beslenme durumu ve riski NRS (Nutritional Risk Screening) 2002, Nutric skoru, MNA (Mini-Nutritional Assessment) ve MUST (Malnutrition Universal Screening Tool) testleri ile değerlendirildi.
Bulgular: Gruplar NRS 2002 (3.37 ± 1.84 vs. 3.93 ± 1.72, p = 0.075), Nutric skoru (4.61 ± 1.85 vs. 4.56 ± 1.85, p = 0.869), MNA (8.0 ± 3.1 vs. 7.1 ± 3.2, p = 0.068) ve MUST skoruna (1.62 ± 1.46 vs. 1.81 ± 1.59, p = 0.456) göre benzer bulundu.
Sonuç: Bu çalışmada, malnütrisyon riski her iki grupta benzer bulundu. Bu sonuç malnütrisyonun diyabete ek olarak eşlik eden diğer hastalıklarla da ilişkili olduğunu düşündürmektedir.

Anahtar kelimeler: Diyabetes mellitus, malnütrisyon, yoğun bakım ünitesi

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INTRODUCTION

Nutrition support in the Intensive Care Unit (ICU) is very important since it has a significant impact on recovery from illness and overall outcome. Patients in the ICU have a higher risk of malnutrition than patients undergoing general admission to hospitals [1]. Malnutrition is associated with poorer clinical outcomes, including postoperative problems, morbidity and mortality [2,3].

Nutritional risks to a patient should be assessed at the time of admission to the ICU, and the enteral nutrition should be started preferably within 24 h [4]. Due to the impaired intake of nutrient and the hypercatabolic-hypermetabolic response to injury or severe illness, severe protein calorie malnutrition is common in patients in the intensive care unit [5]. Types of diseases and treatment modalities can also lead to the exacerbation of malnutrition [6].

Malnutrition is a common public health problem but is generally under-recognized as a health concern in patients [7]. It is also a common problem in elderly patients: depending on the screening and assessment methods used, malnutrition is present in 5%–30% of older adults [7].

Diabetes mellitus (DM) is a common chronic metabolic disease associated with serious complications, demand for multimodal treatment, and significant economic burden [8]. With the development of complications and hospital lengths of stay, life expectancy is worsened with diabetes, and nutritional status is generally correlated with these total outcomes.

The aim of this study was to investigate the nutritional status of patients with diabetes in the ICU of a tertiary hospital.

MATERIAL and METHODS

This retrospective study was conducted in the internal medicine intensive care unit of a tertiary hospital in Turkey from 20 December 2017 to 20 February 2018. The Institutional Review Board approved this study and all procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. Patients treated in the ICU between September 2015 and February 2018 were scanned. One hundred and ninety-two patients were enrolled and divided into two groups. The study group comprised of 77 patients with type 2 diabetes and the control group comprised of 115 patients without diabetes. All patients had co-morbidities such as sepsis, renal failure, respiratory insufficiency, cardiovascular diseases, cerebrovascular diseases, gastrointestinal system diseases, malignancies, etc.

The nutritional risk assessment was tested with NRS (Nutritional Risk Screening) 2002, Nutric score, MNA (Mini-Nutritional Assessment) and MUST (Malnutrition Universal Screening Tool). The NRS-2002 consists of a nutritional score and severity of disease score and an age adjustment for patients aged >70 years. Nutritional score was calculated as follows: weight loss >5% in 3 months or food intake below 50% to 75% in preceding week=1; weight loss >5% in 2 months or BMI 18.5 to 20.5kg/m2 and impaired general condition or food intake 25% to 60% in preceding week=2; and weight loss >5% in 1 month or >15% in 3 months or BMI <18.5kg/m2 and impaired general condition or food intake 0% to 25% in preceding week=3. Severity of disease score: hip fracture, chronic patients with acute complications=1, major abdominal surgery, stroke, severe pneumonia, hematological malignancies=2, and head injury, bone marrow transplantation, intensive care patients with Acute Physiology and Chronic Health Evaluation (APACHE) score >10=3. NRS-2002 score is the total of the nutritional score, and severity of disease score and age adjustment. Patients are classified as no risk=0, low risk=0 to 1, medium risk=3 to 4, and high risk ≥4 [9].

The Mini Nutritional Assessment Screening Form is a nutritional screening tool especially designed for the older population. It consists of 6 questions, scored from 0 to 2 or 3. These questions deal with weight loss, appetite, mobility, psychological stress, neuropsychological problems, and BMI [10].

The NUTRIC score was calculated using age, number of co-morbidities, number of days between admittance to hospital, APACHE II at admittance and the sequential organ failure assessment

(SOFA) score [9].

Age, gender, body mass index (BMI), ICU length of stay, Glasgow coma score, APACHE II, SOFA, invasive mechanical ventilator (IMV) frequency, duration of IMV, NRS 2002, Nutric score, MNA and MUST score, and co-morbidities were recorded.

The MedCalc 18.2.1 software program (MedCalc Belgium) was used for statistical analysis. Data was reported as the mean ± standard deviation. The Kolmogorov-Smirnov test was used to show the normal distribution of quantitative measurements. Chi-square was used to test the statistical significance of differences in gender distribution and malnutrition risk. T test or Mann Whitney U tests were used for comparison of quantitative measurements (age, BMI, Glasgow coma score, APACHE II, SOFA, duration of IMV, NRS 2002, Nutric score, MNA and MUST score) between the two groups. An odds ratio was used to analyze the degree of association between the malnutrition score and diabetes. The probability of making a Type I error (alpha, significance) is 0.05 in all tests.

RESULTS

Table 1 shows the baseline characteristics and comparisons of the study and control groups. The groups were matched in terms of age and gender (p = 0.091, 0.426, respectively). The mean ages of the study and control groups were 68.6 ± 13.4 and 71.6 ± 14.6 years old, respectively. There were 38 women and 39 men in the study group, and 46 women and 69 men in the control group. Patients with diabetes had higher BMIs compared to patients without diabetes (28.7 ± 7.5 vs. 24.4 ± 4.4, p<0.001). Intensive care unit length of stay was comparable in diabetic and non-diabetic patients (10.6 ± 10.3 vs. 8.9 ± 7.6 days, p = 0.422, respectively, table 1). There was no statistically significant difference between the groups according to the invasive mechanical ventilator (IMV) frequency and duration of IMV (p=0.243, p=0.06, respectively, table 1).

The percentages of co-morbidities for each group are shown on table 2. The Glasgow coma score, APACHE II and SOFA of the both groups were comparable (p > 0.05, for each, table 2).

Frequencies of malnutrition risk in patients with diabetes according to the NRS 2002, Nutric score, MNA and MUST score were 77.9%, 53.2%, 41.6%, 66.2%, respectively while they were 87.8%, 55.7%, 55.7%, 67.8% in patients without diabetes. There were no difference between the groups according to the malnutrition risk frequencies (p=0.586, 0.810, 0.264, 0.918, respectively, table 2).

Table 1. Demographical characteristics of the groups.

	DM (+) N=77	DM (-) N=115	p
Age (years)	68.6 ± 13.4	71.6 ± 14.6	0.091
Female N (%)	38 (49.4%)	46 (40.0%)	0.426
BMI kg/m^2	28.7 ± 7.5	24.4 ± 4.4	<0.001
IMV frequency	31 (40.3%)	33 (28.7%)	0.243
Duration of IMV	10.8 ± 13.5	6.2 ± 4.2	0.06
Intensive care unit length of stay (days)	10.6 ± 10.3	8.9 ± 7.6	0.422

DM: Diabetes Mellitus, BMI: Body Mass Index, IMV: Invasive mechanical ventilator

Table 2 Comparison of the groups according to the co-morbidities, disease severity and nutrition status

	DM (+) N=77	DM (-) N=115	p
Co-morbidities			
Sepsis	39 (50.6%)	61 (53.0%)	0.855
Respiratory insufficiency	48 (62.3%)	59 (51.3%)	0.424
Renal failure	27 (35.1%)	34 (29.6%)	0.566
Cardiovascular disease	22 (28.6%)	27 (23.5%)	0.543
Cerebrovasculardisease	18 (23.4%)	33 (28.7%)	0.532
Gastrointestinal system disease	6 (7.8%)	14 (12.2%)	0.379
Malignancies	9 (11.7%)	20 (17.4%)	0.351
Disease severity			
Glasgow	12.7 ± 2.5	12.7 ± 2.3	0.760
APACHEII	21.1 ± 6.6	20.5 ±6.3	0.539
SOFA	4.4 ± 2.4	5.0 ± 3.2	0.136
Malnutrition risks			
NRS 2002	60 (77.9%)	101 (87.8%)	0.586
Nutric score	41 (53.2%)	64 (55.7%)	0.810
MNA	32 (41.6%)	64 (55.7%)	0.264
MUST score	51 (66.2%)	78 (67.8%)	0.918
Malnutrition scores			
NRS 2002	3.37 ± 1.84	3.93 ± 1.72	0.075
Nutric score	4.61 ± 1.85	4.56 ± 1.85	0.869
MNA	8.0 ± 3.1	7.1 ± 3.2	0.068
MUST score	1.62 ± 1.46	1.81 ± 1.59	0.456

The groups were comparable according to the NRS 2002 (3.37 ± 1.84 vs. 3.93 ± 1.72, p = 0.075), Nutric score (4.61 ± 1.85 vs. 4.56 ± 1.85, p =

0.869), MNA (8.0 ± 3.1 vs. 7.1± 3.2, p = 0.068) and MUST score (1.62 ± 1.46 vs. 1.81 ± 1.59, p = 0.456, table 2). According to the odds ratio, there was no association between any of malnutrition score and diabetes (p=0.07, 0.742, 0.056, 0.817, respectively, table 3).

DISCUSSION

In this study, we investigated the nutritional status of patients with diabetes in an internal medicine intensive care unit. We tried to show the effect of diabetes on nutritional status in ICU patients. We used nutritional risk assessment tests and we compared both groups according to NRS 2002, Nutric score, MNA and MUST score. A variety of methods were used to evaluate the nutritional situation of patients admitted to hospital. As there is no gold standard of nutritional evaluation, and as most of the methods are inconvenient and time-consuming, they were not routinely used [11]. In most of the previous studies NRS 2002 and MNA are commonly used. Nutric score was not studied for patients with diabetes in the ICU but it was found to be superior to NRS 2002 for assessing malnutrition risk in the patients [12]. It has also better performance than the commonly used MUST score in critically ill patients [13]. According to the current study results, we found comparable nutritional scores in both of the groups with all nutritional risk assessment tests.

Diabetes mellitus is a risk for malnutrition [14] and diabetic patients with malnutrition are at an increased risk of morbidity and mortality. This condition also lowers quality of life and increases the medical costs [15-17]. In this study we found that 77.9%, 53.2%, 41.6%, 66.2% of patients with diabetes were malnourished, according to the NRS-2002, Nutric score, MNA and MUST score, respectively. A few previous studies have investigated malnutrition in patients with diabetes: in Spain, in a prospective observational study, Sanz et al. reported that 21.2% of patients with diabetes were malnourished and accounting for half of the in-hospital deaths. The nutritional evaluation was carried out only with the MNA, within the first 24-72 hours of hospital admission, in their study [16]. In the current study, we have found 41.6% according to the MNA test, though the frequency of malnourished patients was higher in our study since we enrolled only internal medicine ICU patients and these patients had severe diseases. On the other hand, the majorities of the patients were elderly and all had co-morbidities. Frequency of malnutrition in elderly patients in hospital was studied before and the estimated frequency was found to be 29%–61% in an elderly hospital population [15-17].

In this study, the BMI of patients with diabetes was higher than the patients without diabetes. Overweight condition is an indicating factor for the pathogenesis of type 2 diabetes mellitus. Adipose tissue increases insulin resistance and proinflammatory cytokine production (leptin, tumor necrosis factor and interleukin-6), leading to increased fasting blood glucose levels in patients and ultimately inducing type 2 diabetes [18]. In accordance with this link, 15.5% of the malnourished patients with diabetes were reported as obese in the literature [19]. In a multicenter study in Belgium, Vanderwee et al. analyzed 2 329 elderly hospitalized patients for malnutrition. Of these patients 455 (11.9%) were diabetes mellitus. They reported the risk of malnutrition as 43% according to the MNA [20] and this result is similar to ours. Vischer et al. conducted a single center study on 164 (37.2% with diabetes mellitus) in patients in Switzerland. They defined the risk of malnutrition prevalence as 50.5% according to the MNA [21].

Our study had some limitations. Firstly, the retrospective study design may be considered a limitation. Secondly, it would have been beneficial if the groups had been designed homogeneously. Thirdly, the correlation analyses between HbA1c and nutritional scores were not performed and fourthly, a multicenter study with a larger sample size could have given clearer results. On the other hand, according to our scan of the literature, this is the first study that investigated the nutritional status of patients with diabetes in an ICU. Additionally, we checked the patients for malnutrition with four different nutritional assessment tests and finally, the severity of illnesses were comparable in both groups.

In conclusion, the risk of malnutrition was comparable in both groups in our ICU and this result may indicate that traditional screening and

assessment tools could not uniformly identify diabetic patients as malnourished or at nutrition risk, in the context of an ICU. Additionally, our results suggest that the malnutrition may not only be associated with diabetes, but also related to co-morbidities. There may be other considerations for older and inpatient subjects, such as reduced energy requirements, decreased taste and smell sensitivities, decreased appetite, polypharmacy, chronic illness, functional status, social factors, etc. [22].

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Effect of Selective Antegrade Cerebral Perfusion with Moderately Hypothermic Lower Body Circulatory Arrest on Biomarkers Related to Endothelial Function

Antegrad Serebral Perfüzyon ve Distal Ilımlı Hipotermik Sirkülatuar Arrest Tekniğinin Endotel Fonksiyonuna İlişkin Biyobelirteçler Üzerine Etkisi

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ABSTRACT

Aim: This study aims to compare biomarkers related to endothelial function during selective antegrade cerebral perfusion with moderate hypothermic lower body circulatory arrest with that of standard cardiac surgery.

Material and Methods: Thirty-six consecutive patients who underwent selective antegrade cerebral perfusion with moderately hypothermic lower body circulatory arrest at 28°C (study group) for aneurysms of the ascending aorta were prospectively compared with 36 patients who underwent standard cardiac surgery (control group) with conventional cardiopulmonary bypass. Nitric oxide, asymmetric dimethylarginine, hydrogen sulfide and total antioxidant capacity status and lactate levels in blood specimens obtained from the vena cava inferior were studied. Clinical results and biochemical parameters were evaluated.

Results: Biomarkers related to endothelial function were found to be similar between the groups except for asymmetric dimethylarginine. The asymmetric dimethylarginine levels were lower, while lactate levels were significantly higher compared to the control group. When the patients with coronary artery disease were excluded from the analysis to rule out the predominance of coronary artery disease patients in one group as a confounding factor, the asymmetric dimethylarginine levels were found to be similar between the two subgroups.

Conclusion: Low plasma levels of asymmetric dimethylarginine in the study group may have a protective role in endothelial nitric oxide synthesis. When patients with coronary artery disease were excluded from both group, biomarkers related to endothelial function were similar in both groups. We consider that endothelial functions are not affected adversely during short periods of moderately hypothermic lower body circulatory arrest.

Key words: Nitric oxide, asymmetric dimethylarginine, hydrogen sulfide, thoracic surgery.

ÖZ

Amaç: Bu çalışma, antegrad serebral perfüzyon ve distal ılımlı hipotermik sirkülatuararrest tekniğinin endotel fonksiyonuna ilişkin biyobelirteçler üzerine etkisini standart kalp cerrahisi ile karşılaştırmayı amaçlamaktadır.

Materyal ve Metod: Asendan aort anevrizması için 28 ° C' da selektif antegrad serebral perfüzyon ve orta derecede hipotermik alt vücut dolaşım durması uygulanan 36 hasta (standart çalışma grubu), konvansiyonel kardiyopulmoner bypass ile standart kalp ameliyatı uygulanan (kontrol grubu) 36 hasta prospektif olarak karşılaştırıldı. Vena kava inferiordan elde edilen kan örneklerinde nitrik oksit, asimetrik dimetilarginin, hidrojen sülfit ve toplam antioksidan kapasite durumu ve laktat seviyeleri incelenmiştir. Klinik sonuçlar ve biyokimyasal parametreler değerlendirildi.

Bulgular: Endotel fonksiyonuyla ilgili biyobelirteçler, asimetrik dimetilarjinin dışındaki gruplar arasında benzer bulundu. Asimetrik dimetilarjinin düzeyleri düşüktü, laktat düzeyleri ise kontrol grubuna göre anlamlı derecede yüksek bulundu. Kontrol gruptaki koroner arter hastalarının baskınlığını nedeniyle çıkan sonuçlarda kafa karıştırıcı bir faktör olarak düşünülerek koroner arter hastaları analiz dışında bırakıldığında, asimetrik dimetilarjinin düzeylerinin, iki alt grup arasında benzer olduğu bulundu.

Sonuç: Çalışma grubundaki düşük plazma asimetrik dimetilarjinin seviyeleri, endotelyal nitrik oksit sentezinde koruyucu bir role sahip olabilir. Koroner arter hastalığı olan hastalar her iki gruptan da dışlandığında, endotel fonksiyonuna bağlı biyobelirteçler her iki grupta da benzerdi. Bu sebeple antegrad serebral perfüzyon ve distal ılımlı hipotermik sirkülatuar arrest tekniğinin kısa dönemde endotel fonksiyonlarını etkilemediğini düşünmekteyiz.

Anahtar Kelimeler: Nitrik oksit, asimetrik dimetil arjinin, hidrojen sülfit, toraks cerrahisi.

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INTRODUCTION

Selective antegrade cerebral perfusion (SACP) with hypothermic lower body circulatory arrest (HLBCA) is defined as a more effective method than deep hypothermic circulatory arrest for cerebral protection during aortic arch aneurysm operations [1]. Until now, promising neurological results have been obtained using this technique. In recent years, to reduce long cardiopulmonary bypass (CPB) times and to avoid adverse effects of profound hypothermia such as coagulation disorders, elevated inflammatory responses, and end-organ dysfunction, SACP with higher core body temperatures (26 to 28°C) have been introduced [2]. Through the proximal right brachial artery with moderate HLBCA at 26 to 28°C, SACP has been used with favorable clinical and neurological outcomes since 1996 in both our clinical setting and in external healthcare centers [2-5]. However, there is a limited number of studies on lower body ischemia or organ functions during moderate HLBCA. Some reports have shown postoperative elevation of serum hepatic, pancreatic enzymes, creatinine, lactate dehydrogenase and C-reactive protein levels without any clinical evidence of organ dysfunction [4-6].

Pathological conditions in vascular bed such as vasospasm, vasoconstriction, excessive thrombosis, and abnormal vascular proliferation result from endothelial dysfunction. Intact endothelial cells play a pivotal role in the regulation of vascular tone and homeostasis by secreting various active substances [7]. Nitric oxide (NO) is one of the main molecules, and decreased synthesis and release of NO results in endothelial dysfunction. It is involved in various physiological and pathological responses, including smooth muscle relaxation, immune regulation, platelet function, and neuronal transmission. Hypoxia stimulates the inducible form of nitric oxide synthase (NOS) which generates higher concentrations of NO and, in hypoxic conditions, reduced production of endothelium-derived NO, and abundant production of inflammatory cells-derived NO occur [8,9]. On the other hand, reoxygenation following hypoxia leads to oxidative stress which also causes endothelial dysfunction by inhibiting endothelium-derived NOS and scavenging NO with superoxide anion [8,9]. Endothelial dysfunction is also linked to elevated levels of asymmetric dimethylarginine (ADMA), an endogenous NOS competitive inhibitor [8,9]. Another gaseous transmitter is hydrogen sulfide (H2S) which interacts with the NO metabolism and oxidative stress in physiological and pathological situations [10]. In addition, increased levels of reactive oxygen species (ROS) lead to endothelial dysfunction via decrease of NO bioactivity. Therefore, total antioxidant levels are critical for a durable endothelial function [11].

It is well-known that CPB and whole-body systemic ischemia/reperfusion (HCA and reperfusion) cause endothelial dysfunction through activation of neutrophils, release of proteolytic enzymes, and products of oxidative stress in vascular structures [12]. However, there is a limited number of data on endothelial function during SACP with moderate LBHCA, despite favorable outcomes reported in the literature. In the present study, we aimed to investigate biomarkers related to endothelial function (NO, H2S, ADMA and total antioxidant capacity (TAC)) during HLBCA with SACP in patients undergoing aortic surgery, compared to standard cardiac surgery under moderate hypothermia and conventional cardiopulmonary bypass (cCPB).

METHODS

In this prospective study, thirty-six consecutive patients operated for an ascending and arcus aortic aneurysm with SACP with moderate HLBCA (study group, SACP+ HLBCA) and another group of 36 patients with either coronary artery disease (CAD) or heart valve disease operated with cCPB (control group, cCPB) between May 2013 and February 2014 were included. The power analysis was carried out to identify the number of patients in both groups. Patients with a recent (<1 week) myocardial infarction, renal, hepatic dysfunction or failure, and/or aortic dissection were excluded. A written informed consent was obtained from each patient. The study was approved by the Ethics Committee (24th May 2013, no: 5) and conducted in accordance with the principles of the Declaration of Helsinki.

Data including patients' characteristics such as age, sex, body mass index, body surface area, presence CAD, diabetes mellitus, and

left ventricular ejection fraction, hematological parameters such as white blood cell and platelet count, biochemical analysis results such as urea, creatinine, aspartate aminotransferase, alanine transaminase, lactate dehydrogenase, gamma-glutamyl transpeptidase, alkaline phosphatase, and total and indirect bilirubin and operative data such as cardiopulmonary bypass (CPB), cross-clamp, and SACP time and body temperature were analyzed. Body temperature was measured with nasopharyngeal probe during the operation. Postoperative intensive care unit (ICU) and hospital stay, duration of ventilator-dependency and complications were also recorded. At the postoperative sixth hour and third day, urea, creatinine, AST, and LDH values were studied.

Surgical Technique

For the patients in the control group, unilateral SACP technique details of the clinic were described in detail previously [4]. For the patients in the control group, standard cannulation (aortic and right atrial cannulas) and CPB procedures were undertaken with similar anesthesia, cardioplegia and monitoring techniques, except for SACP with HLBCA.

Blood Sampling

Blood samples were drawn from the inferior vena cava to obtain venous blood draining from the visceral organs before termination of SACP which corresponds to the removal of cross-clamping in the cCPB group. Plasma lactate levels were evaluated with blood gas analysis at prespecified time points. Blood samples were taken into tubes containing ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich Inc Schnelldorf, Germany) for further analysis (i.e., NO, H2S, ADMA, and TAC levels). All blood samples were centrifuged with 5.000 rpm for 5 min and the plasma obtained were stored at -80°C until analysis which was performed in a single session.

Laboratory Analyses of NO, H2S, ADMA and TAC

The plasma nitrite levels were measured to assess NO production. The measurement was conducted using the spectrophotometric method based on the Griess reaction [13]. This method was modified at Ankara University, Medical Pharmacology Department for 96-well plates. The plasma TAC levels were measured using the method which was described previously [14], based on the reduction of Cu+2 to Cu+1 by the antioxidants in the plasma. Neocuproine (Sigma-Aldrich Inc, Schnelldorf, Germany) was used as a chromogenic agent and a colored complex was formed spectrophotometrically at 455 nm. The plasma H2S levels were measured spectrophotometrically, according to the previously described method based on the measurements of the absorbance of the methylene blue, which produced by the chemical reaction between N, N-dimethyl-p-phenylenediamine and FeCl3, at 670 nm [15]. The ADMA levels were measured using the enzyme-linked immunosorbent assay (ELISA) kit (Immunodiagnostic A.G., Bensheim, Germany) according to the manufacturer's instruction.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows, version 17.0 software (SPSS Inc., Chicago, IL, USA). The power and sample analysis, version 3.0.43 software was used to identify the sample size. Descriptive data were presented in mean ± standard deviation (SD) and median (min-max) values for continuous variables and in number and frequency for categorical variables. The chi-square or Fisher's exact tests were used to compare categorical variables between the groups. The Student's t-test or Mann-Whitney U test were used for continuous variables in independent groups for parametric and non-parametric variables, respectively. A p value of <0.05 was considered statistically significant.

RESULTS

Patient Characteristics and Operative Data

The patients' characteristics and preoperative hematological and biochemical parameters were similar between the groups, except for incidence of CAD in the cCPB group (19.4% vs. 75%, $p<0.001$) (Table 1). Additional procedures in the SACP+ HLBCA group were aortic valve replacement (n = 7), mitral valve replacement (n = 1), aortic valve replacement + coronary artery bypass grafting (n = 2), and coronary artery bypass grafting (n = 2). In the cCPB group, these procedures were isolated coronary artery bypass grafting (n = 27),

mitral valve replacement (n = 5), aortic valve replacement + mitral valve replacement (n = 1), aortic valve replacement (n = 1), septal myectomy + aortic valve repair (n = 1), and modified Bentall procedure (n = 1). For procedure-related data, the CPB time was longer (p = 0.03) and hypothermia was more pronounced in the SACP+ HLBCA group (Table 2).

Table 1. Patients' characteristics, preoperative hematological and biochemical parameters

Variables	Study group (n=36) mean±SD	Control group (n=36) mean±SD	P value
Age (years)	55.03±15.16	56.94±10.16	0.97a
Female Gender n (%)	7 (19.4%)	15 (41.7%)	0.07b
Mass (kg)	76.36±13.28	78.94±15.87	0.52a
Height (cm)	168.44±14.15	166.42±8.68	0.18a
BMI (kg/m2)	26.54±4.38	28.17±4.89	0.21a
BSA (m2)	1.87±0.20	1.85±0.21	0.73*
CAD n (%)	7 (19.4%)	27 (75%)	<0.001b
DM n (%)	2 (5.6%)	6 (16.7%)	0.26c
EF (%)	56.7±9.1	53.2±8.4	0.13a
WBC (K/mm3)	7164.2±1,734.5	7215.8±1,605.6	0.90a
Plt (K/mm3)		250454.5±62,043.9	0.77a
Urea (mg/dl)	37.89±10.84	37.72±8.10	0.90a
Creatinine (mg/dl)	0.95±0.16	0.92±0.18	0.55d
AST (IU/L)	20.08±5.99	19.89±5.84	0.96a
ALT (IU/L)	21.11±11.88	21.92±10.31	0.83a
LDH (IU/L)	380.08±107.13	364.61±71.36	0.43d
GGT (IU/L)	25.47±12.88	24.91±14.88	0.93d
ALP (IU/L)	76.08±18.59	73.06±25.65	0.97a
Total bilirubin (mg/dl)	0.75±0.45	0.63±0.37	0.16d
Direct bilirubin (mg/dl)	0.20±0.94	0.17±0.86	0.27a

BMI: Body mass index, BSA: Body surface area, CAD: Coronary artery disease, DM: Diabetes mellitus, EF: Ejection fraction, WBC: White blood cell, Plt: Platelet, AST: Aspartate amino transferase, ALT: Alanine amino transferase, LDH: Lactate dehydrogenase, ALP : Alkaline phosphatase, GGT: Gamma glutamyl transferase, SD: Standard deviation, kg: kilogram, cm: centimeter, m: meter, mm: millimeter, mg: milligram, dL: deciliter, IU: International unit. a:Student T test; b: Chi-Square test; c:Fischer's exact test; d:Mann Whitney U test, Study group: Selective antegrade cerebral perfusion (SACP)+Hypothermic lower body circulatory arrest (HLBCA), Control group: Conventional cardiopulmonary bypass (cCPB)

Clinical and Biochemical Outcomes

No mortality was observed in either group. However, the patients in the SACP+ HLBCA group required longer mechanical ventilation support postoperatively in the ICU (11.5±4.3 vs. 9.2±3.1 h, p = 0.01), while the length of ICU and hospital stay and complication rates were similar in both groups (p>0.05) (Table 2). Postoperative clinical and biochemical results are given in Table 2.

Table 2. Intraoperative and postoperative variables between study and control groups

Variables	Study group (n=36) mean±SD	Control group (n=36) mean±SD	P value
CPB period (minutes)	113.2±36.71	95.2±33.59	0.03a
Cross clamp (minutes)	74.64±31.86	62.72±26.57	0.09a
ASCP period (minutes)	14.75±3.71	-	
Temperature (°C)	28.00±0.00	31.36±1.64	0.00a
ICU stay (days)	1.3±0.6	1.3±0.5	0.95a
Hospitalization (days)	6.6±1.5	6.7±1.9	0.43a
Mechanical ventilation (hours)	11.5±4.3	9.2±3.1	0.01b
Complications n (%)	6 (30.6%)	5 (13.9%)	0.16c
Intraoperative			
Lactate (mmol/L)	3.90±0.24	2.9±3.4	
Postoperative 6th hour			
WBC (K/mm3)	11656.7±3,552.0	9961.1±2,493.0	0.02b
PLT (K/mm3)	165666.7±39,240.7		0.47b
Urea (mg/dl)	37.9±10.7	34.5±10.0	0.23b
Creatinine (mg/dl)	1.0±0.2	1.0±0.2	0.32b
AST (IU/L)	51.0±25.6	72.7±56.4	0.20b
LDH (IU/L)	692.2±163.4	740.8±300.5	0.92b
Postoperative 3rdday			
WBC (K/mm3)	10459.1±3,957.1	9691.9±2881.0	0.41b
PLT (K/mm3)	162888.9±51006.3		0.12b
Urea (mg/dl)	42.7±15.7	38.7±13.1	0.25b
Creatinine (mg/dl)	0.9±0.3	0.9±0.2	0.32a
Total Bilirubin (mg/dl)	0.8±0.4	0.7±0.5	0.83a
Direct Bilirubin (mg/dl)	0.3±0.2	0.3±0.2	0.97a
AST (IU/L)	45.3±21.9	52.9±29.1	0.25a
ALT (IU/L)	26.3±30.1	35.7±47.1	0.78a
LDH (IU/L)	643.1±188.0	700.3±225.4	0.43a
ALP (IU/L)	66.4±16.7	75,3±32.6	0.21b
GGT (IU/L)	36.4±27.7	45.7±42.7	0.76a

CPB: Cardiopulmonary bypass, ASCP: Antegrade selective cerebral per-

fusion ,ICU: Intensive care unit, WBC: White blood cell, Plt: Platelet, AST: Aspartate amino transferase, ALT: Alanine amino transferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, SD: Standard deviation, kg: kilogram, cm: centimeter, mm: millimeter, mg: milligram, dL: deciliter, IU: International unit, L: liter, a: Mann Whitney U test ; b:Student T test; c:Fischer's exact test, Study group: Selective antegrade cerebral perfusion (SACP)+Hypothermic lower body circulatory arrest (HLBCA), Control group: Conventional cardiopulmonary bypass (cCPB)

The WBC counts of the SACP+LBHCA group were higher than cCPB group at the postoperative sixth hour (11656.7±3552.0 vs. 9961.1±2493.0 K/mm3, p = 0.02), but returned to normal limits at the postoperative third day (10459.1±3957.1 vs. 9691.9±2881.0 K/mm3, p = 0.41). Other blood test results were similar at the postoperative third day in both groups. Postoperative complications were observed six patients in SACP+ HLBCA group whereas five patients in cCPB group (p = 0.16). Postoperative new onset atrial fibrillation developed in one case in SACP+ HLBCA group and three cases in cCBP group.

Blood Sample Analysis

A statistically significant difference was observed in terms of the ADMA and lactate levels between the groups. Lactate levels were higher (3.90±0.24 vs. 2.9±3.4 mmol /L) and the ADMA levels were lower in the SACP+ HLBCA group (1.30±0.42 vs. 1.62±0.35 µmol/L /L) than cCPB group (<0.001). (Table 2, 3). However, TAC, NO, and H2S levels were similar between the groups (Table 3).

Table 3. Comparison of ADMA, TAC, H2S and NO levels vena cava inferior blood samples and their relevant P values in the study and the control group before and after the patients with coronary artery disease are excluded (Subgroups 1 and 2).

Variables	ADMA (µmol/L)	TAC (µmol/L)	H2S (mM)	NO (µmol/L)
Study group(n=36) mean±SD	1.30± 0.41	814.61± 198.87	30.33± 12.40	48.01± 46.26
Controlgroup (n=36) mean±SD	1.62± 0.36	782.54± 214	28.89± 5.98	71.11± 96.70
P value	<0.001a	0.512b	0.476a	0.604a
Subgroup 1 (n=29) mean±SD	1.30± 0.43	798.26± 202.4	30.10± 13.6	52.55± 49.5
Subgroup 2 (n=9) mean±SD	1.46± 0.33	793.14± 207.1	26.73± 5.6	61.39± 92
P value	0.121a	0.877a	0.355a	0.953a

CAD: Coronary artery disease, ADMA: Asymmetric dimethylarginine, TAC: Total antioxidant capacity; H2S: Hydrogen sulfide, NO: Nitric oxide; SD: Standard Deviation; a:Man Whitney U test; b: Student T test, Study group: Selective antegrade cerebral perfusion (SACP)+Hypothermic lower body circulatory arrest (HLBCA), Control group: Conventional cardiopulmonary bypass (cCPB), Subgroup 1: Study group without coronary artery disease patients, Subgroup 2: Control group without coronary artery disease patients

As high ADMA levels were reported to be associated with atherosclerosis in a previous study [9], a further analysis was performed to exclude patients with CAD from both groups in our study. Study group without CAD patients defined as Subgroup 1 and control group without CAD patients defined as Subgroup 2. The resultant twenty-nine and nine patients in the Subgroup 1 and Subgroup 2, respectively were compared again. The mean values of TAC, NO, H2S, and ADMA levels were found to be similar in both groups (p>0.05).

DISCUSSION

In the present study, in patients with SACP with moderately HLBCA, when patients with coronary artery disease were excluded from both groups we found no statistically significant difference in biomarkers related to endothelial function (NO, H2S, ADMA and TAC), biochemical parameters, and clinical status, compared to the control group.

Deep HCA has been questioned in recent years due to its undesirable systemic effects, such as coagulopathy, increased systemic inflammatory response, higher renal and respiratory failure, neuronal injury, and cerebral microvasculature endothelial dysfunction [12,16]. In a recent meta-analysis, it was found to be associated with higher stroke rates, compared to moderately HCA with SACP [17]. In the past, experimental studies and series were more concerned with time for neuronal injury than time for visceral tissue injury in aortic surgery, as neuronal tissues have a lower threshold for ischemic injury [18]. It is usually not recommended to exceed 30 min of arrest period during HCA without SACP (12 to 15°C). However, with SACP and mild-to-moderate HCA technique, the safe limit of circulatory arrest period for visceral organ protection in the lower body has not been well-established, yet. A mean HLBCA period of 14.8±3.7 min in the study group is a relatively short timeframe to observe any visceral complication at 28°C; however, even with such brief periods, higher lactate levels were observed in the SACP+ HLBCA

group than the cCPB group (3.9±1.5 vs. 2.2±0.8 mmol /L, p< 0.001). This finding suggested that non-oxidative phosphorylation began within this period of moderately HCA in the SACP+ HLBCA group, compared to the control group whose lower bodies were perfused at the meantime. However, we observed no clinical or biochemical alteration in the postoperative period between these groups. In some clinical experimental studies, it was also indicated that the safe limit of circulatory arrest time can be 60 min during moderate hypothermia to avoid visceral ischemia/reperfusion injury and to diminish systemic inflammatory response [16, 19]. Indeed, the present study was planned in such a way that blood samples would have been drawn every 15 min of HLBCA. However, the longest HLBCA period was 28 min in only one patient in the study group. Hence, it still remains unclear whether longer periods of LBHCA may lead to considerable alterations.

Pacini et al. emphasized the importance of temperatures higher than 25°C to be an independent protective factor for isolated liver dysfunction [20]. In accordance with the above mentioned study, no evidence of visceral organ injury was observed in the SACP+ HLBCA group which was performed under moderate hypothermia (28°C). During postoperative follow-up, we observed no clinical impairment of hepatic, renal, or neurological functions, except for longer mechanical ventilation support time in the HLBCA group. This difference can be attributed to the established practices of the ICU specialists about late extubation of aortic arch surgery patients in the ICU. On the other hand, the length of postoperative stay, hospitalization, and complication rates were similar in both groups.

Blood temperature during CPB was shown to be an important indicator of NO production in an experimental study [21]. In the aforementioned study, NO production was higher in a tepid temperature (34°C) than more hypothermic CPB temperatures (28°C). During circulatory arrest and reinstitution of circulation, endothelial hypoxia and subsequent reoxygenation induce oxidative stress with enhanced superoxide generation and diminished NO production, leading to endothelial dysfunction, which is named as ischemia/reperfusion injury [22]. In addition, it is known that, under conditions of hypoxia and acidosis, endothelial NOS activity diminishes, thereby leading to decrease NO levels [23]. These factors may account for the slightly lower NO levels in the study group, although it did not reach statistical significance. The difference might have been more pronounced for longer antegrade cerebral perfusion duration in the present study.

The ADMA levels should be evaluated together with NO levels. The ADMA inhibits the synthesis of NO competitively and plays a crucial role in the initiation of endothelial dysfunction [9]. This close relationship between the ADMA and NO has been studied in a number of studies [24]. In our opinion, this study indicates that low plasma levels of endogenous NO synthase inhibitor ADMA in the aneurysms group during short periods of moderately HLBCA may have a protective role in endothelial NO synthesis. We also know that chronic serum ADMA level increase is to related CAD. In the present study, the ADMA levels were lower in the study group and higher in the control group. As the number of patients with cCAD was higher in the control group, we decided to exclude patients with CAD from both groups to rule out the predominance of CAD patients in one group as a confounding factor. When patients with cCAD were eliminated, we observed no difference regarding ADMA levels between the two subgroups.

Hydrogen sulfide has been identified on vascular endothelium as the third endogenous signaling gasotransmitter (after NO and CO) which has many biological functions such as metabolic modulation, vasodilatation, and angiogenesis [10,25]. Moreover, H2S exerts powerful antioxidative, anti-inflammatory, cytoprotective, and organ-protective effects, particularly in kidneys [26-28]. While physiological concentrations of H2S have cytoprotective effects, its high concentrations are associated with cytotoxic effects, as H2S itself is already toxic [10]. In addition, H2S plays a critical role for the persistence of the endothelial system function [29]. Many experimental studies have shown protective effects of H2S on liver, kidney, lung, and heart after ischemia/reperfusion injury [26,30]. Plasma concentration of H2S, therefore, may be a good indicator for endothelial function after ischemia/reperfusion injury. Furthermore, H2S also works as an antioxidant owing to its thiol

group that allows reduction of disulfide bonds and radical scavenging [10]. In the present study, we found no significant difference between the groups in terms of the H2S levels. This result may indicate that short periods of HLBCA (14 min), as in our study, may affect endothelial function not more than cCPB.

The amount of ROS is expected to increase during moderately hypothermic HLBCA as a result of reperfusion injury. The level of antioxidant components of plasma is of utmost importance for the continuity of endothelial function, since ROS can damage cells and lead to endothelial dysfunction [11]. In the present study, there was no statistically significant difference in the TAC levels between the two groups. On the other hand, higher lactate levels in the study group suggested that anaerobic glycolysis began early during HLBCA due to insufficient oxygen supply. In addition, similar serum TAC levels indicated that HLBCA had no additional harmful effect on the antioxidative status during this short period of ischemia, compared to cCPB.

Limitations

Nonetheless, the present study has some limitations. First, ischemic time (SACP+ HLBCA period) of the study group was most probably short to observe any endothelial dysfunction. Long duration of SACP with moderately HLBCA was not desired by surgeons to avoid clinical complications in aortic surgery. Although the study was originally designed to compare longer HLBCA times as well, we observed no complex arch pathologies that would require longer circulatory arrest periods for a durable and complete repair. Therefore, the longest SACP time was 28 min in only one patient in the study group. Second, it would have been more appropriate to compare this technique with other aortic surgery techniques, such as deep HCA. However, we have mostly abandoned the use of such techniques in our clinic and use it on rare occasions, such as pediatric patients who are considered ineligible candidates. Third, the number of patients with cCAD in the control group exceeded that of in the study group. When these patients were excluded for subgroup analysis, the resultant patient numbers in both subgroups were not similar, indicating in a weak statistical comparison. Fourth, Blood samples for analysis (i.e., NO, H2S, ADMA, and TAC levels) were drawn from the inferior vena cava to obtain venous blood draining from the visceral organs before termination of SACP which corresponds to the removal of crossclamping in the cCPB group. However, if the sampling had been drawn after initial reperfusion, blood level tests would have been more meaningful for endothelial function. Finally, cCPB temperature and cCPB periods were longer in the study group due to the more complex nature of the aortic surgery technique, compared to other operations in the control group.

CONCLUSION

In conclusion, despite these limitations, we consider that endothelial functions are not affected adversely during short periods of moderately HLBCA. Low plasma levels of ADMA, endogenous NO synthase inhibitor, in the study group may have a protective role in endothelial NO synthesis. When patients with CAD were excluded from both groups to rule out the predominance of CAD patients in the control group as a confounding factor, biomarkers related to endothelial function were found to be similar in both subgroups during short periods of HLBCA. This favorable result is also true for clinical results, compared to other cardiac operations with cCPB. However, further large-scale studies are needed to confirm our findings and to investigate visceral organ status in longer periods of SACP with moderately HLBCA.

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A comparison of a new diagnostic test of the human Brucellosis, the Brucella Coombs Gel Test, with other methods

Brusellozun Tanısında Yeni Bir Metot Olan Brucella Coombs Gel Testin Diğer Yöntemlerle Karşılaştırılması

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ABSTRACT

Aim: The aim of this study was to compare the efficiency of the Brucella Coombs Gel Test (BCGT), a new serological diagnosis test, with the methods used in routine laboratory such as Brucella immuncapture test (BCAP), Standard Tube Agglutination (STA), Rose Bengal (RB) and ELISA for the diagnosis of brucellosis.
Patients and Method: The serum samples of 107 patients with a presumptive diagnosis of brucellosis sent from three different clinics (internal medicine, infectious disease and pediatric clinics) were subjected to four different diagnostic methods (BCAP, RB, STA, and ELISA). The correlations between these diagnostic tests were analyzed using the Cohen's Kappa test. Additionally, sensitivity, specificity, positive and negative predictive values, and accuracy of BCGT were measured.
Results: According to the obtained data, the positivity of different Brucella tests (BCAP, RB, STA, BCGT, ELISA IgG and IgM) were 102 (95.3%), 96 (89.7%), 80 (74.8%), 100 (93.5%), 104 (97.2%) and 101 (94.3%), respectively. According to the Kappa test results, there was strong agreement between BCAP and BCGT (K=0.824). Furthermore, the sensitivity and specificity values of BCGT in our study were 98.08% and 71.43%, respectively.
Conclusion: BCGT is a rapid, cost-effective and highly sensitive test, which appears to be a promising technique for the diagnosis of human brucellosis; however, further scientific studies are needed to support the applicability of this test in routine laboratories.

Keywords: Brucella, Coombs Gel test, Rose Bengal, Standard tube agglutination

ÖZ

Amaç: Bu çalışmada, rutin laboratuvarlarda brusellozun tanısında kullanılan Brucella immuncapture test (BCAP), Rose Bengal (RB), Standart Tüp Aglütinasyon (STA) ve ELISA testleri ile yeni bir serolojik test olan Brucella Coombs Gel Test (BCGT)'in etkinliğinin karşılaştırılması amaçlanmıştır.
Hastalar ve Yöntem: Dâhiliye, enfeksiyon hastalıkları ve pediatri kliniklerinden bruselloz ön tanısı ile laboratuvarımıza gönderilen 107 hastaya ait serum örneklerinde BCAP, RB, STA, ELISA IgG/IgM ve BCGT testleri uygulanmıştır. Cohen's Kappa testi ile tanı testleri arasındaki uyum istatistiksel olarak analiz edilmiştir. Ayrıca, BCGT testinin duyarlılık, özgüllük, pozitif ve negatif prediktif değerleri saptanmıştır.
Bulgular: Elde edilen verilere göre; 107 hastanın 102'si (95.3%) BCAP testi ile 96'sı (%89.7) RB testi ile 80'i (%74.8) STA testi ile 100'ü (%93.5) BCGT ile 104'ü (%97.2) ELISA IgG testi ile ve 101'i (94.3%) ELISA IgM testi ile pozitif olarak tespit edilmiştir. Yapılan istatistiksel analizler sonucunda (Cohen's Kappa Test), BCAP ile BCGT (K=0.824) arasında güçlü uyum saptanmıştır. Ayrıca BCGT testinin duyarlılık ve özgüllüğü sırasıyla %98.08 ve %71.43 olarak saptanmıştır.
Sonuç: BCGT insan brusellozunun tanısında kullanılabilecek umut vadeden bir teknik gibi görünen, hızlı, az maliyetli ve yüksek duyarlılığa sahip bir testtir. Ancak, bu testin rutin laboratuvarlarında kullanılabilirliğini destekleyecek daha fazla bilimsel çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Brusella, Coombs Gel test, Rose Bengal, Standart tüp aglütinasyon

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INTRODUCTION

Brucellosis is a zoonotic infection transmitted from animals to humans by ingestion of infected food products, direct contact with infected animals or inhalation of aerosols [1, 2]. Serious difficulties are still encountered in understanding the pathogenic mechanisms of human brucellosis, identifying the markers that indicate the severity, progression of disease, and developing treatment methods [3, 4].

Diagnosis of human brucellosis is quite important because of the long treatment period. Despite the long incubation period (minimum 7 days), blood culture is the gold standard method for brucellosis, however, serological methods have been used more frequently due to the difficulties in the growth and identification of microorganisms [5]. The Rose Bengal (RB) is a rapid, high-sensitivity, cost-effective plaque agglutination test. Furthermore, it is frequently used as a screening test in human brucellosis; however, it should be evaluated together with other verification methods [6]. Standard tube agglutination (STA) test is also an easy, inexpensive and reliable test in the diagnosis of brucellosis, when evaluated together with the clinical data, and it is the most commonly used method in the serologic diagnosis of brucellosis in the world [7], as total antibodies against S-lipopolysaccharide on the bacterial surface are detected. It should be noted, however, that this test has some disadvantages such as being time-consuming and laborious and leading to false negative results because of the blocking of antibodies [8]. On the other hand, the ELISA method enabled us to detect more positivity as well as different classes of antibodies than other agglutination methods. In this method, different results can be obtained depending on the structure of the solid phase and anti-globulin, which affects the sensitivity, specificity and applicability of the method, the antibody profile may not always be clinically compatible and titers may remain positive for a long time. Furthermore, ELISA tests are more expensive than other agglutination methods, requiring experienced personnel [5]. In recent years, the Brucella immuncapture test (BCAP), which is based on sandwich ELISA, has been used to detect blocking antibodies. In the BCAP test, the wells were coated with Coombs antibodies developed against human IgG, IgM, IgA, and three antibodies can be detected in the serum of the patient. Additionally, these tests have been defined as a useful methods for both diagnosis and follow-up the disease [7, 9, 10].

In the laboratory diagnosis of brucellosis, new studies are needed to establish new diagnostic tests with high sensitivity/specificity, are cost-effective and provide reliable results in a short period [11]. The Brucella Coombs Gel Test (BCGT) is developed in our country and is a new method that is being used in the serological diagnosis of brucellosis. In this test, tube agglutination and the coombs method are performed together in gel wells and its most important advantage is that it provides results in 30 minutes and does not require incubation [12].

The aim of this study was to compare the efficiency of BCGT, a new serological diagnosis test, with other diagnostic methods used in routine laboratory such as BCAP, STA, RB and ELISA.

PATIENTS AND METHOD

The serum samples of 107 patients with a presumptive diagnosis of brucellosis sent from three different clinics (internal medicine, infectious disease and pediatric clinics) to Kafkas University, Health Research and Application Hospital, Microbiology Laboratory between January 2015-2016 were included in the study. The local ethics committee of Kafkas University, Faculty of Medicine approved the study (Approval number: 218).

In our microbiology laboratory, BCAP, RB, and STA tests were routinely performed to suspected patients' sera. BCGT and Brucella ELISA IgM/IgG tests were then performed to our experimental group's sera. The Brucella IgG/IgM antibody test kits were used in accordance with the manufacturer's protocols.

The Brucella Coombs test antigen, which is routinely used in our laboratory, has a smooth lipopolysaccharide (LPS) structure. The Brucella immuncapture test (Metser Lab, Istanbul, Turkey) was also used in accordance with manufacturer's protocols. For evaluation, the blue/purple dot shape was evaluated as negative and homogeneous

turbidity above 1/160 was evaluated as positive.

The procedure of studying of BCGT: The first well of plate was filled with 5-µL serum+100-µL diluent, and other wells were filled with only 50-µL diluent. Brucella antibody was added to the serum samples, which were diluted on 96-well plates. The samples were transferred to the 12x8 gel matrix microtubes including antihuman IgG gel matrix (Coombs antibodies). The microtubes were centrifuged for 20 minutes at 3,000 rpm. Then, the results were evaluated visually (negative if pink colored was seen at the bottom of microtubes, and positive if pink colored was seen on the top of the microtubes).

The Brucella IgG and IgM ELISA tests were performed and interpreted according to the manufacturer's instructions (Vircell, S.L., Spain). Briefly, 96-well microplate were coated with 100 µl of Brucella antigen. After the steps of incubation and washing 100 µl of 1:1000 dilution of serum was added and microplates were then incubated. The following processes were completed and the reaction was finally stopped. The results were read on a MultiSkan GO spectrophotometer (Thermo Fisher Scientific) at 450 nm absorbance. The results obtained via the five (BCAP, RB, STA, BCGT, ELISA) methods were recorded.

The results of the BCGT were compared with the Brucella immuncapture test, and the sensitivity, specificity, positive and negative predictive values of the BCGT were calculated. The total number of cases examined (TN), true positive (TP), falsa positive (FP), true negative (TN), and false negative (FN) results were used for calculation. The sensitivity and specificity are equal to TP/(TP+FN), and TN/(TN+FP), respectively. Accuracy was calculated as the proportion of the true results (both true positives and true negatives) among the total number of cases examined [13]. All obtained data were analyzed by using the Statistical Package for the Social Sciences (SPSS) version 22.0 software (SPSS Inc., Chicago, IL, USA). The "number (n)," "percentage (%)," "mean," "standard deviation (SD)," median, minimum and maximum values were given for the descriptive statistics. The independent samples t-test or Mann-Whitney U test were used to compare numerical variables. Agreements of the diagnostic tests of Brucella were calculated by Cohen's Kappa coefficient. The kappa values between 0.21 and 0.40 were interpreted as fair, 0.41–0.60 as moderate, 0.61–0.80 as good, and >0.80 as almost perfect agreement [14].

RESULTS

Of the 107 patients who attended three different clinics (infectious disease, internal medicine and pediatric), 57 were men (53.3%) and 50 were women (46.7%). The mean age was 40.29±13.64. There was no statistically significant difference between age and gender (Z=-1.625, p=0.104). The distribution of patients according to departments was internal medicine clinic (n=62, 57.9%), infectious disease clinic (n=35, 32.7%) and pediatric clinic (n=10, 9.3%), respectively. The characteristics of the patients are reported in Table 1.

Table 1: Demographic data of patients

Patient characteristics	Male (n, %)	Female (n, %)	Total
Age	42.72±12.61	37.52±14.35	40.29±13.64
Gender	57 (53.5%)	50 (46.7%)	107
Clinics			
Internal medicine	32 (56.1%)	30 (60.0%)	62
Infectious disease	21 (36.8)	14 (28.0%)	35
Pediatric	4 (7.0%)	6 (12.0%)	10

According to the obtained data, the positivity of different Brucella tests (BCAP, RB, STA, BCGT, ELISA IgG and IgM) was 102 (95.3%), 96 (89.7%), 80 (74.8%), 100 (93.5%), 104 (97.2%) and 101 (94.3%), respectively. The detailed positivity and negativity rates are shown in Table 2.

Table 2: Distribution of positive and negative results of Brucella diagnostic tests

		Positive (n, %)	Negative (n, %)
RB		96 (89.7)	11 (10.3)
STA		80 (74.8)	27 (25.2)
BCGT		100 (93.5)	7
ELISA	IgG	104 (97.2)	3 (2.8)
	IgM	101 (93.5)	6 (6.5)
BCAP		102	5

RB: Rose Bengal, STA: Standard Tube Agglutination, BCGT: Brucella Coombs Gel Test, BCAP: Brucella Capture Test

Furthermore, the agreements among Brucella diagnostic tests were analyzed by Cohen's Kappa test. According to the Kappa test results, there were an excellent/almost perfect agreement between BCAP and BCGT (K=0.824). A moderate agreement was detected between BCAP and ELISA test (K=0.482). In contrast, there were no significant agreement by Kappa test between other Brucella diagnosis methods. The Cohen's Kappa test results are shown in Table 3.

Table 3: The Kappa test results of Brucella diagnostic tests

	STA	RB	BCGT	ELISA	BCAP
STA		κ= -0,171	κ= 0,344	κ= 0,157	κ= 0,254
RB	κ= -0,171		κ= -0,087	κ= -0,046	κ= -0,069
BCGT	κ= 0,344	κ= -0,087		κ= 0,375	κ= 0,824
ELISA	κ= 0,157	κ= -0,046	κ= 0,375		κ= 0,482
BCAP	κ= 0,254	κ= -0,069	κ= 0,824	κ= 0,482	

RB: Rose Bengal, STA: Standard Tube Agglutination, BCGT: Brucella Coombs Gel Test, BCAP: Brucella Capture Test

On the other hand, the sensitivity and specificity of BCGT compared to BCAP is reported in Table 4. After the calculations, the sensitivity and specificity of BCGT were 98.08% [95% CI: 93.23-99.77%] and 71.43% [95% CI: 29.04-96.33%], respectively. The accuracy was calculated according to the formula given in the statistical analysis part of this study and it was detected as 96.4% [95% CI: 91.03-99.01%].

Table 4: The sensitivity and specificity values of Brucella Coombs Gel Test

	BCGT	CI 95%
Sensitivity	98.08%	93.23% - 99.77%
Specificity	71.43%	29.04% - 96.33%
Positive Likelihood Rate	3.43	1.06 – 11.07
Negative Likelihood Rate	0.03	0.01 – 0.13
Positive Predictive Value	98.08%	87.44% - 97.43%
Negative Predictive Value	71.43%	36.96% - 91.42%
Accuracy	96.4%	91.03% - 99.01%

BCGT: Brucella Coombs Gel Test. The calculations were performed by using Brucella Capture test as a gold standard test

DISCUSSION

The diagnosis of brucellosis in clinic is quite difficult especially in the absence of specific clinical features. At that point, specific microbiological tests should be used to diagnose the probable brucellosis in order to prevent the problems associated with the treatment processes and responses [15-17]. These diagnostic tests are the Rose Bengal test [18], the standard tube agglutination test [10], the immuncapture test [19] and the ELISA test [20]. In our study, the results have shown that the BCGT test is a promising technique for the diagnosis of human brucellosis, is in agreement with gold standard test and has high sensitivity (98.08%).

A literature check has revealed similar results. A study (n=117) reported positive results in 81 (95.3%) patients with Rose Bengal, 53 (62.3%) patients with STA and 64 (75.3%) patients with Coombs test [21]. Another study from Turkey (n=71) reported positive results in 56 (%78.8) patients with Rose Bengal, 30 (42.2%) patients with STA and 52 (73.2%) patients with BCAP [5]. We found 89.7% positivity with RB, 74.87% positivity with STA, 93.5% positivity with BCGT, and 97.2% positivity with ELISA IgG. On the other hand, a study that compared the diagnosis methods (Brucella Coombs Gel test and STA) reported 100% positivity rate in BCGT [10].

The sensitivity rates of BCGT compared to the Brucella immuncapture test reported in scientific studies from Turkey are between 94-100%, and the specificity rates are between 82-100% [10, 21]. Kalem et al. [22] performed a study that compared both ELISA and Coombs Gel tests in a similar fashion as in our study. They reported that BCGT and ELISA tests had high sensitivity (100% and 92.8%, respectively) and specificity (100% and 79.7%, respectively) when compared to the immuncapture test. Another study reported that the sensitivity, specificity, positive and negative predictive values of the Coombs gel test were 100%, 82.2%, 84.3%, and 86%, respectively [23]. The results were closely similar; however, only a study reported [16] that the sensitivity of BCGT was 78.8%, if the titer was above 1/160, and the accuracy of this test was 88.7%. In our study, we compared the positivity results of BCGT to BCAP. The sensitivity and specificity were 98.08%, and 71.43%, respectively and in addition, the accuracy was determined to be 96.4%.

On the other hand, the kappa test results supported

the sensitivity and specificity rates of BCGT results. A study performed in 2015 [24] reported that BCGT was in excellent agreement with the Brucella immuncapture test (K=0.979). Another study [5] stated similar findings as with others, that BCGT was in almost perfect agreement with classic coombs test (K=0.846), though Koçman et al. [16] detected that the BCGT (above 1/160) was in good agreement with Brucella immuncapture test (K=0.724). In our study, we detected that there was an excellent agreement between the BCGT and BCAP tests with the following Kappa test findings: K=0.824. Eventually, this high sensitivity/specificity rates and high agreement results of the BCGT compared with other diagnosis test makes it more preferable. Most of the related studies reported similar percentages and Kappa results with the BCGT and advised to use it in the diagnosis of brucellosis [10, 17, 23].

Study Limitations:

Our study had some limitations, namely that the study population should be extended to get better results about the sensitivity and specificity of diagnostic tests. Secondly, the blood culture results and follow-up of the patients should be obtained and included in further studies.

CONCLUSION

As a result, it is now well established that the Brucella Coombs Gel is a high sensitive, cost-effective and rapid test compared to the ELISA and PCR methods; however, more comprehensive studies should be performed in both control groups and patients, in order to show the realistic efficiency of the BCGT and to support the applicability of this test in routine laboratories.

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Morphometric evaluation of coccyx in patients with coccydynia and classification

Koksidinialı Hastalarda koksiksin morfometrik değerlendirilmesi ve klasifikasyonu

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ABSTRACT

Aim: This study aimed to aimed to classiffication the morphological and pathological anatomic features of patients with coccydynia.
Patients and Method: Patients over 16 years of age who were admitted to Alanya Alaaddin Keykubat University Educational Research Hospital with complaints of coccydynia between April 2015 and April 2018, were included in the study. The sitting and standing coccyx and anterior-posterior pelvic radiographs of the patients were examined retrospectively.
Results: It was observed that coccyx scoliosis, hypermobility, and dislocation were unrelated to sacrococcygeal angulation. There was a positive correlation between sacrococcygeal and intercoccygeal angles in the measurements of sitting and standing coccyx radiographs of the patients. There was a correlation between the length of the sacrum and length of the coccyx and also the intercoccygeal angle and coccyx length in the unoperated patients. There was a positive correlation between length of the sacrococcygeal joint and sacrococcygeal angle in males. Fusion in the sacrococcygeal joint was less in females.
Conclusion: In the evaluation of patients with coccydynia, it should not be forgotten that the coccyx is a spinal segment which can move in all planes and develop pathology. Side and AP positioned sitting and standing dynamic radiography is an effective method for the detection of pathology in this patients. New classification including pathology in all three planes is needed in the classification of patients with coccydynia. We believe that our classification will eliminate this deficit.

Keywords: coccydynia, coccyx, classification, Morphology

ÖZ

Amaç: Koksidinalı hastalarda morfolojik ve patolojik anatominin özellikleri sınıflandırmayı amaçladık.
Hastalar ve Yöntem: Nisan 2015 - Nisan 2018 tarihleri arasında Alanya Alaaddin Keykubat Üniversitesi Eğitim Araştırma Hastanesine koksidina yakınması ile başvuran 16 yaş ve üzerindeki hastalar çalışmaya dahil edildi. Hastaların oturarak ve ayakta çekilen koksiks ve ön-arka pelvis grafileri retrospektif olarak incelendi.
Bulgular: Koksiksin skolyozunun, hipermobilitesinin ve dislokasyonunun sakrokoksigeal açılanma ile ilişkili olmadığı görüldü. Hastaların oturarak ve ayakta çekilen koksiks grafi ölçümlerinde sakrokoksigeal ve interkoksigeal açıları arasında pozitif yönlü anlamlı bir ilişki bulundu. Ameliyat olmamış hastalarda sakrum ve koksiks uzunluğu arasında ve interkoksigeal açı ve koksiks uzunluğu arasında ilişki bulundu. Erkeklerde sakrokoksigeal eklem uzunluğu ile sakrokoksigeal açı ilişkili bulundu. Kadınlarda sakrokoksigeal eklemde füzyonun daha düşük olduğu tespit edildi.
Sonuç: Koksidina yakınması ile gelen hastalar değerlendirilirken koksiksin tüm düzlemlerde hareket edebilen ve patoloji geliştirebilen bir omurga segmenti olduğu unutulmamalıdır. Bu hastaların yan ve AP pozisyonda oturarak ve ayakta çekilen dinamik direkt grafilerle değerlendirilmesi patolojinin bulunmasında etkili bir yöntemdir. Koksidinalı hastalarının sınıflandırılmasında her üç düzlemde patoloji içeren yeni sınıflandırma gereklidir. Sınıflandırmamızın bu eksikliği gidereceğini düşünmekteyiz.

Anahtar Sözcükler: Koksidinia, Koksiks, Sınıflandırma, Morfoloji

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INTRODUCTION

The coccyx is a conical, distal-most section of the spine, usually consisting of 3-4 segments. Although the sacrococcygeal junction may rarely fuse, it usually remains mobile for life [1,2].

Coccydynia was first used by Simpson in 1859 to describe pain surrounding the coccyx exacerbated by sitting [1-3]. Its etiology includes sitting for long periods of time, falling, traumas that may occur during birth or spinal surgery, and less frequently, chordoma, intraductal schwannoma, perineural cysts, giant-cell tumor, and intraosseous lipoma. However, cases where the cause is not found are also quite frequent [1-3]. Conservative methods such as use of donut-shaped cushions, rest, medical treatment, physical therapy, massage, radiotherapy, psychotherapy, sacral rhizotomy, manipulation, epidural injection, and local injection have been successfully applied in the treatment of coccydynia [1-4]. Coccygectomy surgery is performed in patients who do not benefit from conservative treatment methods [3,5].

Direct coccyx radiography along with computed tomography (CT), three dimensional CT, and magnetic resonance imaging (MRI) is used to evaluate patients with coccydynia, which is more frequently observed in women than in men. However, direct radiography is frequently used for evaluation due to its fast results, ease of access, low price, and easy dynamic filming [6-9].

It has been reported that the morphological and pathoanomical features of the coccyx are important in the development of coccydynia. Clinical studies use the Postacchini-Massobrio classification, which is based on the angle of the sagittal plane between the sacrum and the coccyx, and revised in 2010 to assess the coccyx. The movement and hypermobility of the coccyx's sagittal, coronal, and horizontal plane may lead to coccydynia [1,2,10-12]. In this study, we aimed to classiffication the morphological and pathological anatomic features of patients with coccydynia.

PATIENTS AND METHODS

In our study, patients aged 16 years or older who were admitted to Alanya Education and Research Hospital between April 2015 and April 2018 with complaints of coccydynia were screened retrospectively. Patients with clear imaging of sitting and standing, side direct coccyx radiography, and direct posteroanterior pelvis radiography were included in the study. Patients with tumors in the coccyx region and patients who had previously undergone surgery in this region for another reason were excluded from the study. Our work was approved by the Ethics Committee of Alaaddin Keykubat University (date 06.07.2018 number 2 decision 8).

On the direct coccyx radiographs of the patients, sacrum length, coccyx length, sacrococcygeal joint length, sacrococcygeal angle and intercoccygeal angle were measured. The number of coccyx segments and presence of fusion in the sacrococcygeal joint were evaluated. Additionally, intercoccygeal and sacrococcygeal angles were measured for scoliosis, and degree of dislocation in patients with dislocation was measured with dynamic radiography. For dynamic radiography, direct side coccyx radiographs of the patients were taken in standing position and after 15 minutes of standing, in an upright sitting position (Figure 1a,b). The angle between the midpoint of the sacrum's upper endplate and the midpoint of the upper endplate of the first coccyx segment (cocx 1), and the angle between the line drawn from there to the end of the coccyx was evaluated as sacrococcygeal angle (Figure 2a). The angle between the line drawn from the first cocx midpoint to the second cocx rear lower corner and the line drawn from this point to the extreme end of the coccyx was evaluated as the intercoccygeal angle (Figure 2b). On the anterior-posterior pelvic radiography, the angle between the line drawn from the midpoint of the upper endplate of the sacrum to the midpoint of the upper endplate of cocx 1 and the line drawn from this point to the tip of the coccyx was evaluated as the scoliosis angle (Figure 3). Patients who underwent surgery were identified among these patients.

Statistical Analysis

All measurements were evaluated by anatomists using the Winsoft RIS / PACS Ver.2.2.39 imaging server. IBM SPSS for Windows version 20.0 software program (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Correlation between sacrum and coccyx length, between intercoccygeal angle and coccyx length, between joint length and sacrococcygeal angle, and between sacrum and coccyx length and intercoccygeal angle of patients who were and weren't operated were evaluated by Pearson correlation tests. Correlation between number of segments and angle types and the relationship between non-operative status and angle types were assessed with the Chi Square test. The t-test was used to determine whether there was a significant difference between intercoccygeal angle and sacrococcygeal angle and number of segments. Mann-Whitney U test was used to evaluate whether there was a significant difference between patients with and without coccyx retroversion, coccyx retroversion and gender, coccyx length, intercoccygeal angle, number of segments, number of sacrum, sacrococcygeal angle length, and length of coccyx in patients with and without scoliosis. P-value of less than 0.05 was considered statistically significant.

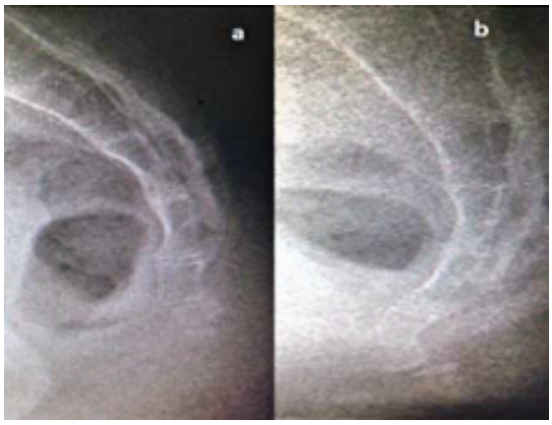

Figure 1. Dislocation of the distal posterior coccyx that is unevident in side coccyx radiography taken standing (a) is visible in radiography taken sitting (b)

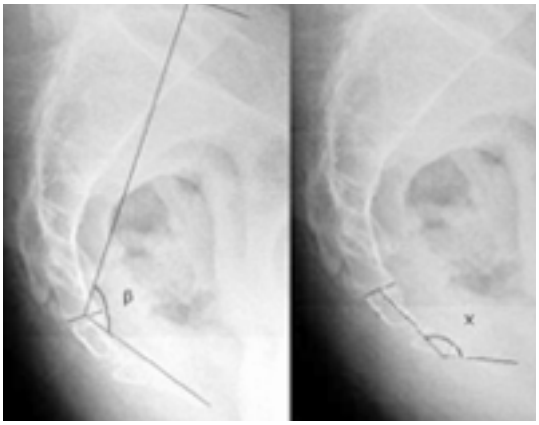

Figure 2. (a) Sacrococcygeal angle β, (b) Intercoccygeal angle x

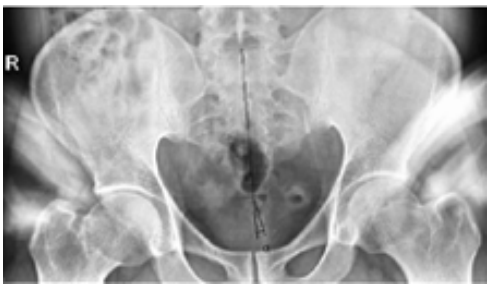

Figure 3. The region marked α is assessed as the scoliosis angle

RESULTS

A total of 53 patients were male (34.2%) and 102 were female (65.8%). The mean age of all patients was 36.7 years (range 16 to 77 years), the average age of the female patients was 37 years (range 16 to 77 years), and the average age of the male patients was 35.8 years (range 16 to 63 years). The distributions of mean, standard deviation, median, minimum and maximum values of coccyx length, sacrum length, fusion age, sacrococcygeal joint length, sacrococcygeal angle and intercoccygeal angle according to gender are provided in Table 1. There was a positively low correlation between sacrum length and coccyx length ($p = 0.004$). Regarding the relationship between sacrum length and coccyx length in males, there was a moderately positive correlation ($p = 0.02$). There was no significant relationship between sacrum and coccyx length in women ($p = 0.07$). Regarding relationship between intercoccygeal angle and coccyx and sacrum lengths, only a significant relationship between intercoccygeal angle and coccyx length was found in males ($p=0.04$). The sacrococcygeal and intercoccygeal angles measured in the sitting and standing coccyx graphs of the patients are provided in Table 2. There was a positive correlation between sitting and standing sacrococcygeal ($p=0.01$) and intercoccygeal ($p=0.01$) angles.

Of the patients participating in the study, 57 (36.8%) had three and 98 (63.2%) had four segments. Of the participating males, 18 (34%) had three, 35 (66%) had four segments, and of the females, 39 (38.2%) had three and 63 (61.8%) had four segments.

Eighteen of our patients underwent coccygectomy surgery. When the operated patients were

classified according to Postacchini-Massobrio classification, it was observed that type 1 was most common, followed by type 2 and 6. There was no significant relationship between the presence or absence of surgery and angle type (p = 0.47).

Regarding the correlation between sacrum and coccyx length and intercoccygeal angle in operated and unoperated patients, there was only a positive relationship between coccyx length and sacrum length in unoperated patients (p=0.03). There was a positive correlation between intercoccygeal angle and coccyx length (p=0.01), however there was no significant correlation between intercoccygeal angle and sacrum length (p=0.25). There was no correlation between coccyx length, sacrum length, and sacrococcygeal angle in operated patients.

When correlation of sacrococcygeal joint length was evaluated according to gender, it was determined that the joint lengths of males were higher than females (p = 0.001). There was a significant relationship between joint length and sacrococcygeal angle in males (p = 0.019), but this difference was not significant in females (p = 0.754). There was no difference between joint length and intercoccygeal angle according to male (p = 0.862) or female (p = 0.118) gender.

There was no significant difference between male and female patients regarding presence of coccyx retroversion (p = 0.41). The mean, standard deviation, median, minimum and maximum values of sacrococcygeal angle and intercoccygeal angle of patients with and without retroversion are given in Table 2. There was also no significant correlation according to presence of coccyx retroversion and coccyx length (p=0.39), intercoccygeal angle (p = 0.19), sacrococcygeal angle (p = 0.72), segment number (p = 0.68), and sacrum length (p = 0.76). There was no significant different between joint lengths of patients with and without coccyx retroversion (p = 0.68).

Of the patients with scoliosis, 54% had right and 46% and left deviation. The mean, standard deviation, median, minimum, and maximum values of right and left deviations according to the genders of the scoliosis angle are given in Table 1. There was no significant difference between patients with and without scoliosis in terms of coccyx length (p = 0.09), sacrococcygeal angle (p = 0.57) and joint length (p = 0.29).

The rate of patients with forward slipping of the sacrococcygeal joint was 44% and the rate of backward slipping was 56%. The mean forward listhesis of the sacrococcygeal joint was 6,81mm (±2,12mm) and mean backward listhesis of the sacrococcygeal joint was 6,52 mm (± 1,88mm). The fusion rate was 24.5% for males and 9.8% for females. The average age of females with fusion in the sacrococcygeal joint was 43.1 years, while the average age of males was 36.5 years. There was no significant difference between patients with and without sacrococcygeal joint fusion, in terms of sacrococcygeal angle (p = 0.48), intercoccygeal angle (standing) (p = 0.38), intercoccygeal angle (sitting) (p = 0.71), sacrum length (p = 0.78), and sacrococcygeal joint length 0.82).

DISCUSSION

Postacchini and Massobrio [11] suggested that coccyx morphology played a role in the etiology of coccydynia. Maigne et al. identified anterior subluxation and emphasized the importance of coccyx hypermobility in coccydynia [13,14]. Kim and Suk [15] found that the scoliotic deformity of the coccyx could cause coccydynia. In a study by Nathan et al. [12] coccyx scoliosis and retroversion were also included in the Postacchini-Massobrio classification. [10] According to the Postacchini-Massobrio classification [10], type 1 coccyx was most common. [3,16] Some studies state that type 2 [3,6] and other state that type 1 [17] most commonly requires coccygectomy. In our study, type 1 coccyx was most common among patients with coccydynia and that type 1 most frequently required coccygectomy. When the types were compared, it was observed that type 1 was operated more frequently due to the higher amount; however there was no statistically significant difference between the types.

In our study, significant differences were observed in sacrococcygeal and intercoccygeal angles in the sagittal plane of coccyx side radiographs taken sitting and standing. Dynamic radiography showed that the coccyx which changed position on the sagittal plane could also change position on the horizontal or coronal planes in the same patient (Figure 1). A coccyx with angulation of the sagittal plane could have scoliosis in the coronal

Table 1.

	Total			Female			Male		
	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max
Coccyx length	38.9±9.4	38.9	23.5-71.3	38.1±8.2	39	23.5-57.7	40.8±11.2	38.5	26.5-71.3
Sacrum length	129.3±14.9	130.7	89-169.7	128.6±15.5	128.6	89-167.7	130.7±13.9	133	89.0-156.8
Age of fusion	36.4±15.2	35.0	16-77	43.1±20.6	39.5	19-77	36.5±15.7	37	16-62
Sacrococcygeal joint length	9.6±2.4	9.6	3.7-16.4	9.2±2.4	9.2	3.7-16.4	10.5±2.1	10.2	7.10-16
Intercoccygeal angle	141.1±17.1	143.5	76.8-168.8	143.9±14.7	146.7	86.8-168.8	135.8±20.3	136.1	76.8-168.2
Sacrococcygeal angle	121.5±20.0	120.5	71.3-192	119.3±21.3	117.9	71.3-192	125.7±16.7	128.3	93.7-164.4
Right scoliosis	8.1±3.7	6.7	4.5-14.5	9.4±3.7	10.1	4.9-14.5	4.8±4.9	4.85	4.5-5.2
Left scoliosis	7.1±2.8	7.3	3.1-12.4	6.7±1.0	7.3	5.6-7.4	7.4±3.8	7.0	3.1-12.4

Mean, standard deviation, median, minimum, and maximum values of coccyx length, sacrum length, age of fusion, length of sacrococcygeal joint, sacrococcygeal angle, intercoccygeal angle, and right and left scoliosis according to gender

Table 2.

	Sacrococcygeal angle						Intercoccygeal angle					
	standing			sitting			standing			sitting		
	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max
No retroversion	129.6°± 32.1°	114.7°	93.7°-186.6°	130.9°± 40.1°	110.4°	91°-194.7°	130.5 ±20.6	132.1°	86.8°-164°	124.4 °±26.9°	120.4°	85.4°-185.6°
Retroversion	180.1°± 18.9°	185.6°	177°-192°	186.8°± 12.4°	189.7°	181.3°-195.3°	155.7° ±11.1°	152.2°	143.5°-168.8°	159.1 ±23.7	150.3°	140.6°-197.1°

.Mean, standard deviation, median, minimum, and maximum values of sacrococcygeal and intercoccygeal angles of patients with and without retroversion

plane, or dislocation in the horizontal plane, and this is unrelated to the sacrococcygeal angle. For the coccyx which can be displaced in all three planes, we believe that a new classification is needed in the classification of patients with coccydynia (Table 3).

In an MRI study of coccyx morphology by Tetiker et al. [8] conducted with 456 healthy Turkish individuals with a mean age of 43.9 found the average coccyx length to be 35.6 mm. This value was evaluated as 38.5 mm for males and 34.5 mm for females, and males were longer than females. Lee et al. [7] found average coccyx length in males to be 37.9 mm and 34.4 mm in females. Indiran et al. [18] found average coccyx length as 33.8 mm in males and 31.5 mm in females. In our patients, we found average coccyx length of all patients to be 38.9 mm, 40.8 mm in males, and 38.1 mm in females. Of all studies, including ours, coccyx length was longer in males than in females as consistent to the literature.

In studies performed with multislice CT, MRI, and three-dimensional CT assistance, the sacrococcygeal angle of males was found to be higher than in females, and significant differences were found in comparison between males and females [5,6,8,15,16]. In our study, the sacrococcygeal angle of males was also higher than females. Previous studies have also evaluated intercoccygeal angles as slightly higher in males than in females. In our study, the intercoccygeal angle was found to be higher in females than in males, consistent with the literature.

It has been found that dynamic radiography of the coccyx taken sitting and standing is necessary to access hypermobility of the coccyx and that coccyx mobility is higher in patients with high body mass index. It is believed that stable imaging is inadequate for the assessment of the coccyx, a dynamic structure which constitutes one of the three

points used in the action of sitting [13,15,19,20]. In our study, significant difference was observed in intercoccygeal and sacrococcygeal angles between sitting and standing radiography.

Table 3.

Type	Morphologic classification of coccydynia
I	No angulation or slight forward inclination of the coccyx
I a	Dislocation
I b	Scoliosis
I c	Hypermobility
II	Angulation less than 90° of the sacrococcygeal joint or coccyx segments and sharp curvature forward
II a	Dislocation
II b	Scoliosis
II c	Hypermobility
III	Angulation greater than 90° of the sacrococcygeal joint or coccyx segments
III a	Dislocation
III b	Scoliosis
III c	Hypermobility
IV	Retroversion
IV a	Dislocation
IV b	Scoliosis
IV c	Hypermobility

Classification of coccydynia including pathologies of all three planes

A study conducted with MRI to determine the number of coccyx segments reported the coccyx was most frequently composed of three vertebra segments (42.1%), while another study conducted with multislice CT reported the coccyx was most frequently composed of four vertebra segments [8,18]. In our patients, coccyx was composed of three segments in 36.8% of the patients, and four segments in 63.2%. We believe this was due to MRI and CT having better imaging of bone, soft tissue, and joints. The literature does not report significant relationship between number of segments and sacrococcygeal angle [9]. We also did not find a relationship between sacrococcygeal angle and number of segments in all of our patients.

A study on healthy individuals reports sacrococcygeal joint fusion in 23.8% of males and 21.6% of females [8]. In our patients, fusion rate was 24.5% in males and 9.8% in females. We believe that coccydynia occurring more frequently in females than males may be due to less frequent sacroccocygeal joint fusion in females.

Studies on coccyx retroversion have defined the sacrococcygeal angle as the angle between the first segment of the coccyx and the fifth segment of the sacrum [9]. We believe the angulation of the farthermost point of the coccyx to be clinically relevant and that this point should be included in measurement. Imaging of the patients showed us that dislocations among coccyx segments may occur along with retroversion evident with sitting (Figure 1). This also leads us to believe that side radiography of the coccyx taken while standing, MRI, or three-dimensional CT is inadequate in assessing the patient, and that side and posteroanterior dynamic coccyx radiography taken sitting and standing may be effective in evaluation of the idiopathically considered or normal anatomically positioned coccyx [15,20].

A three-dimensional CT study on the Korean population found the mean right deviation of patients with scoliosis was 12.0° in females, 13.1° in males, and for left deviation, 15.2° in females and 13.1° in males [6]. In our study, mean right deviation was 9.4° in females and 4.8° in males, and in left deviation, 6.7° in females and 7.4° in males. These results led us to believe a difference due to racial factors.

In our study, correlation between sacrum and coccyx length and between intercoccygeal angle and coccyx length was found in unoperated patients. This suggests that individuals with anatomically compatible sacrum and coccyx lengths and compatible coccyx length and intercoccygeal angle are less operated.

Sacrococcygeal joint length was found to be longer in males and a relationship was found between sacrococcygeal joint length and sacrococcygeal angle. We believe that this is related to the fact that the operation frequency is less in males.

Limitations: Dynamic coccyx radiographies in healthy patients could compared with coccydynia patients but this was not done because of ethical rules.

CONCLUSION

The coccyx is a spinal segment that moves on three planes and may develop pathology. Dynamic direct side and posteroanterior radiography taken sitting and standing is an effective method to

determine pathology in patients with coccydynia. We believe that a new classification including patology in all three planes is needed in the classification of patients with coccydynia therefore, our classification will eliminate this deficit.

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Effect of astaxanthin in imatinib mesylate-induced cardiotoxicity

İmatinib Mesilat Kaynaklı Kardiyotoksisitede Astaksantinin Etkileri

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ABSTRACT

Aim: Imatinib mesylate is a tyrosine kinase inhibitor and is approved as a standard first-line therapy of chronic myeloid leukemia. Oxidative stress, as well as intracellular calcium overload and mitochondrial dysfunction, play an important role in chemotherapy-induced cardiotoxicity. The underlying pathophysiological mechanism associated with imatinib-induced cardiotoxicity is not well understood. In the present study, we investigated alterations in calcium influx, oxidative stress and apoptosis through transient receptor potential melastatin 2 (TRPM2) channels. Also, we aimed to investigate if there is a modulator role of astaxanthin in cardiomyocytes during imatinib mesylate-induced cardiotoxicity.

Materials and methods: The cells were divided into seven main control groups: imatinib, imatinib+antranilic acid, imatinib+astaxanthin, imatinib+antranilic acid+astaxanthin, astaxanthin and astaxanthin+antranilic acid groups. Cells in the groups were stimulated with cumene hydroperoxide and inhibited with antranilic acid in related experiments for activation and inactivation of TRPM2 channels, respectively. We measured cytosolic calcium, intracellular reactive oxygene, mitochondrial depolarization, caspase 3 and caspase 9 levels.

Results: The apoptosis values were significantly lower in the astaxanthin and the imatinib+astaxanthin group than in the imatinib group of cardiomyocytes ($p< 0.001$). The cell viability values were significantly higher in the imatinib+astaxanthin+antranilic acid ($p<0.001$) and the imatinib+astaxanthin ($p<0.05$) groups, than in the imatinib group.

Conclusions: As a result, we found that TRPM2 channels were found in cardiomyocyte cells and they were activated by reactive oxygen species. Also, we showed that overactivated TRPM2 channels are associated with increased cytosolic free calcium, oxidative stress and apoptotic cell injury in imatinib mesylate-induced cardiotoxicity, whereas astaxanthin could have a modulator role in this instance.

Keywords: İmatinib mesylate, TRPM2, oxidative stress, cardiomyocyte

ÖZ

Amaç: İmatinib mesilat bir tirozin kinaz inhibitörüdür ve kronik miyeloid löseminin standart bir birinci basamak tedavisi olarak onaylanmıştır. Kemoterapiye bağlı kardiyotoksisitede oksidatif stresin yanı sıra hücre içi kalsiyum aşırı yüklenmesi ve mitokondriyal disfonksiyon önemli rol oynar. İmatinib ile indüklenen kardiyotoksisitenin neden olduğu altta yatan patofizyolojik mekanizma tam olarak anlaşılamamıştır. Bu çalışmada, geçici reseptör potansiyel melastatin 2 (TRPM2) kanalları üzerinden kalsiyum akışı, oksidatif stres ve apoptozdaki değişimleri araştırdık. Ayrıca, imatinib mesilat kaynaklı kardiyotoksisite sırasında astaksantinin kardiyomiyositlerde modülatör rolü olup olmadığını araştırdık.

Gereç ve Yöntemler: Hücreler, kontrol, imatinib, imatinib + antranilik asit, imatinib + astaksantin, imatinib + antranilik asit + astaksantin, astaksantin ve astaksantin + antranilik asit grupları olmak üzere yedi ana gruba ayrıldı. Gruplardaki hücreler, ilgili deneylerde TRPM2 kanallarının aktivasyonu ve inaktivasyonu için sırasıyla kümen hidroperoksit ile uyarıldı ve antranilik asit ile inhibe edildi. Sitosolik kalsiyum, hücre içi reaktif oksijen, mitokondriyal depolarizasyon, kaspaz 3 ve kaspaz 9 seviyeleri ölçüldü.

Bulgular: Apoptoz değerleri astaksantin ve imatinib + astaksantin grubunda, imatinib grubundaki kardiyomiyositlerden anlamlı olarak daha düşüktü ($p<0.001$). Hücre canlılığı değerleri imatinib + astaksantin + antranilik asit ($p<0.001$) ve imatinib + astaksantin ($p <0.05$) gruplarında imatinib grubundan anlamlı olarak daha yüksekti.

Sonuç: Sonuç olarak, TRPM2 kanallarının kardiyomiyosit hücrelerinde bulunduğunu ve reaktif oksijen türleri ile aktive edildiğini bulduk. Ayrıca, aşırı aktifleştirilmiş TRPM2 kanallarının, imatinib mesilat ile indüklenen kardiyotoksisitede artmış sitosolik serbest kalsiyum, oksidatif stres ve apoptotik hücre hasarı ile ilişkili olduğunu, buna karşın astaksantinin bu aşamada modülatör bir rol oynayabileceğini gösterdik.

Anahtar kelimeler: İmatinib mesilat, TRPM2, oksidatif stres, kardiyomiyosit

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INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disease which develops from a translocation between chromosomes 9 (Abl) and 22 (Bcr). The mutant Bcr_Abl protein (active tyrosine kinase) can induce malignancies and is associated with CML [1]. Imatinib mesylate is a tyrosine kinase inhibitor and it is approved as a standard first-line therapy of CML [2]. Imatinib mesylate-induced cardiotoxicity including left ventricular dysfunction and heart failure has been shown before in previous studies [3,4]. In addition to limited clinical data on cardiotoxicity rates, imatinib-induced cardiac dysfunction is difficult to diagnose in early stages. Also, the underlying pathophysiological mechanism associated with imatinib-induced cardiotoxicity is not well understood. It is thought that imatinib is one of the cardiotoxic agents affecting mitochondrial function [5].

Antioxidant therapies have been evaluated in clinical trials with patients at risk of cardiovascular events. In most studies, antioxidants have failed to show any cardiovascular benefits since patients are not selected based on the presence of confirmed oxidative stress. Astaxanthin (xanthophyll carotenoid) is accepted as a potent antioxidant and with its anti-inflammatory properties, it has potential as a therapeutic agent in cardiovascular disease [6]. As the involvement of mitochondrial dysfunction and oxidative stress in cardiotoxic responses have been shown, we decided to examine the effects of imatinib on calcium signaling, apoptosis, mitochondrial depolarization levels and oxidative stress in cardiomyocytes. Also, we wanted to evaluate if there is a modulator effect of astaxanthin through TRPM2 channels in imatinib-induced cardiotoxicity.

MATERIALS AND METHODS

Reagents

Caspase-3 substrate (AC-DEVD-AMC) and Caspase-9 substrate (AC-LEHD-AMC) were provided from Enzo (Lausen, Switzerland). Dulbecco's modified Eagle's medium, Trypsin–EDTA, Fetal Bovine Serum and penicillin-streptomycine and Dimethyl sulfoxide, Dihydrorhodamine-123 (DHR 123) were provided from Sigma-Aldrich (St. Louis, MO), Fura-2 (AM) calcium florescent dye was bought from Calbiochem (Darmstadt, Germany). APOPercentage assay with releasing buffer were purchased from Biocolor (Belfast, Northern Ireland). Pluronic® F-127 was obtained from Biovision (San Francisco, USA). A mitochondrial stain 5,50, 6,60-tetrachloro-1,10,3,30-tetraethylbenzimidazolyl carbocyanine iodide (JC-1) and Probenecid were obtained from Santa Cruz (Dallas, Texas, USA). MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from Thermo Fischer (Waltham, MA, USA).

Cell culture

AC16 (Human cardiomyocyte cell line) was obtained from ATCC. Cardiomyocytes were cultured in DMEM containing 10% fetal bovine serum. Cardiomyocytes were seeded in 8-10 flasks at a density of 1×106 cells per flask. Cells were incubated in T25 flasks at 37°C at 5% CO2 in a humidified incubator. After cells have reached 75–85% confluence, cells were incubated with the chemical compounds described in the groups section. Cells were examined daily for evidence of contamination and after treatments, the cells were detached with %0.25 Trypsin EDTA for analysis and split into the sterile falcon tubes for analysis.

Study Groups

Cardiomyocytes were cultured at 37°C and divided into seven main groups.

Group 1 (Control): Cardiomyocytes were not incubated with Imatinib (IMTNB), Antranilic Acid (ACA) and Astaxanthin (ASTX) but were kept in a flask in the same condition for 72 h.

Group 2 (IMTNB): Cardiomyocytes were incubated with 50 µM Imatinib for 24 hrs.

Group 3 (IMTNB+ACA): Cardiomyocytes were incubated with 50 µM Imatinib for 24 hrs and then incubated with Antranilic acid (ACA, 0.04 mM, 30 min).

Group 4 (IMTNB+ASTX): Cardiomyocytes were incubated with 50 µM Imatinib for 24 hrs and then incubated with 40 µM Astaxanthin for 12 hrs.

Group 5 (IMTNB+ASTX+ACA): Cardiomyocytes

were incubated with 50 μM Imatinib for 24 hrs, then incubated with 40 μM Astaxanthin for 12 hrs and then incubated with Antranilic acid (ACA, 0.04 mM, 30 min).

Group 6 (ASTX): Cardiomyocytes were incubated with 40 μM Astaxanthin for 12 hrs.

Group 7 (ASTX+ACA): Cardiomyocytes were incubated with 40 μM Astaxanthin for 12 hrs and then incubated with Antranilic acid (ACA, 0.04 mM, 30 min).

In related experiments (except for calcium signaling), the cells were farther treated with Cumen hydroperoxyde (CMPx) (0.1 mM, 10 min) for activation of TRPM2 channel before related analysis and they were also inhibited the TRPM2 blocker ACA (0.04 mM, 30 min) before related analysis during 1.2 mM extracellular calcium. During calcium signaling analysis (Fura-2/AM), cells were stimulated on 20th cycles with 0.1 mM CMPx in the existence of 1.2 mM Calcium and calcium free buffer in extracellular environment.

Evaluation of intracellular free calcium concentration ([Ca2+]i)

After cell were incubated with the chemical compounds described in the groups section, cells were detached with %0.25 Trypsin–EDTA from T25 flasks, then centrifuged (100G, 5 min). The medium was taken off and changed with HEPES-buffered saline [HBS; 5 mM KCl, 145 mM NaCl, 10 mM D-glucose, 1 mM MgCl2, 1 mM CaCl2, (1.2 mM). 10 mM HEPES and 0.1% (w/v) BSA; pH 7.4] containing 5 μM Fura-2 AM (Ca2+-sensitive fluorescent ratiometric dye) and 0.05% (w/v) Pluronic F-127 and cells were incubated for 1 hour at 37˚C in the dark. The loaded cardiomyocyte cells were washed twice with HBS and covered with 1 ml of HBS supplemented with 2.5 mM probenecid for at least 20 minutes at 37˚C in the incubator (in the dark) to allow for Fura-2 AM de-esterification. Cells were seeded in clear flat-bottom 96-well (black) culture trays (Grainer Cell Star, Life Sciences USA) at a density of 3×104 cells/each well. Fluorescence emission intensity at 510 nm was determined in individual wells using a plate reader equipped with an automated injection system (SynergyTM H1, Biotek, USA) at alternating excitation wavelengths of 340 and 380 nm every 3 seconds for 50 acquisition cycles (cycle: 3 s; gain: 120) in response to agonists (CMPx, 0.1 mM) added by the automated injector. [Ca2+]i in cells was expressed as the average emission at 510 nm in each wells in response to excitation at 340 nm / 380 nm normalized to initial fluorescence emission obtained during the first 20 cycles. Measurement of [Ca2+]i was performed according to the previous study [7].

Evaluation of intracellular reactive oxygen species production

DHR-123 (Dihydrorhodamine 123) is a cell-permeable nonfluorescent reactive oxygen species (ROS) indicator can easily pass the cell membranes where it is oxidized to cationic rhodamine 123 which localizes in the mitochondria and exhibits green fluorescence. The Rh123 fluorescence intensities were determined by the previously described method using an automatic microplate reader (SynergyTM H1, Biotek, USA). Excitation and emission wave lengths of the analyses were 488 nm and 543 nm, respectively [8].

Assay for programmed cell death (apoptosis), caspase-3 / -9 activities

The apoptosis assay was executed with a commercial kit by Biocolor Ltd. (Northern Ireland) that according to the manufacturer instructions as method previously described [8,9]. The detection of apoptosis by spectrophotometry (SynergyTM H1, Biotek, USA) was achieved at 550 nm.

The activities of caspase-3 and -9 were measured by the previously described method [10]. The cleavages of caspases' substrates were determined by the microplate reader (SynergyTM H1, Biotek, USA) with 360 nm (excitation wavelength) and 460 nm (emission wavelength). The apoptosis and caspase values were given as fold change over the pre-treatment level.

Analyses of the Potential of Mitochondrial Membrane

The potential of mitochondrial membrane was determined by a cationic fluorescent dye which can pass through the cell membrane. This dye accumulates in the normally respiring mitochondria and reduction the red-to-green fluorescence

intensity in the medium shows mitochondrial depolarization. The membrane potential changes were evaluated by previously described method [11]. JC-1 fluorescence was measured by a single excitation wavelength (488 nm) with dual emission [green (520 nm)and red (596 nm)] using the microplate reader (SynergyTM H1, Biotek, USA). JC-1 values were assessed as fold change relative to untreated control cells.

The potential of mitochondrial membrane was determined by a cationic fluorescent dye which can pass through the cell membrane. This dye accumulates in the normally respiring mitochondria and reduction the red-to-green fluorescence intensity in the medium shows mitochondrial depolarization. The membrane potential changes were evaluated using the previously described method [11]. JC-1 fluorescence was measured by a single excitation wavelength (488 nm) with dual emission [green (520 nm)and red (596 nm)] using the microplate reader (SynergyTM H1, Biotek, USA). JC-1 values were assessed as fold change relative to untreated control cells.

Cell viability (MTT) assay

The MTT method for measuring the mitochondrial activity of living cells was used in this study to demonstrate the effects of IMTNB and ASTX on cell viability. Cardiomyocytes were incubated with the chemical compounds described in the Experimental Design Section and then performed by previously described method by an automatic microplate reader (SynergyTM H1, Biotek,USA) [12].

Statistical analyses

All results were presented as means ± standard deviation (SD). Differences between groups were analyzed with one-way ANOVA. Statistical analyses were calculated using GraphPad Prism version 7.04 for windows (GraphPad Software, San Diego California, the USA). P<0.05 was considered significant.

RESULTS

Effects of imatinib and astaxanthin on cytosolic calcium levels in cardiomyocytes: The effect of imatinib and astaxanthin administrations on cytosolic calcium levels in cardiomyocyte cells are shown in figure 1A / 1B. The TRPM2 channel antagonist antranilic acid (ACA) was used in in-vitro model to evaluate the receptors involved in Ca2+ increase through TRPM2 channels. As shown in figure 1B, the Ca2+ concentration in cardiomyocytes was higher in the imatinib than in the control (p<0.001). The Ca2+ concentration was lower in the astaxanthin+ACA compared to the control (p<0.05). Also, cytosolic Ca2+ concentration was lower in the imatinib+ACA, imatinib+astaxanthin and imatinib+astaxanthin+ACA than in the imatinib (p<0.001). In addition, in cytosolic Ca2+ concentration, there is no statistically significant difference between imatinib+astaxanthin+ACA compared to the imatinib+astaxanthin group.

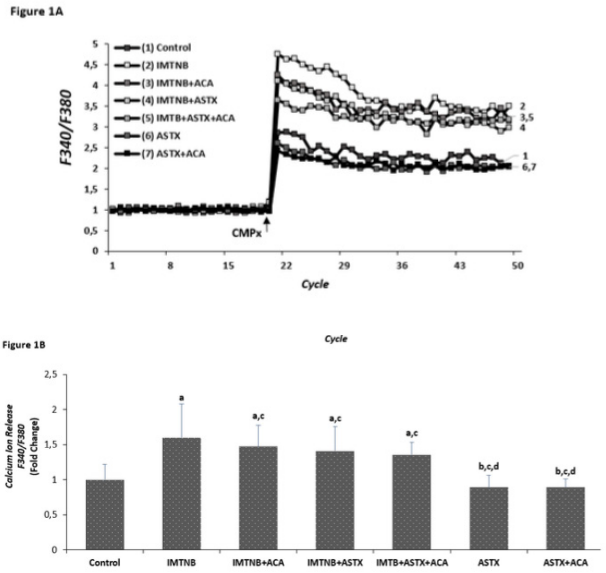

Figure 1A / 1B . The effect of Imatinib (50 µM, 24 hrs) and Astaxantin (40 µM, 12 hrs) on cytosolic calcium levels in cardiomyocyte cells. Cardiomyocytes are stimulated by Cumene hydroperoxide (CMPx 0.1 mM and on 20th cycle) but they were inhibited with Antranilic Acid (ACA 0.1 mM for 30 min) (mean ± SD and n=3). ap<0.001 and bp<0.05 vs control, cp≤0.001 vs IMTNB, dp<0.001 vs IMTNB+ASTX and fp<0.05 vs ASTX .

Effects of imatinib and astaxanthin on apoptosis, ROS and MTT (Cell Viability) levels in cardiomyocytes

Effects of imatinib and astaxanthin administrations on apoptosis levels are shown in figure 2A. The values were higher in the imatinib group than in the control group (p<0.001). The apoptosis values were significantly lower in the astaxanthin and the imatinib+astaxanthin than in the imatinib group of cardiomyocytes (p<0.001). Also, the values were significantly lower in the imatinib+astaxanthin+ACA

when compared with the imatinib+astaxanthin (p<0.001).

Intracellular ROS production of groups are shown in figure 2B. The ROS production values were higher in the imatinib group than in the control (p<0.001). The values were significantly lower in the imatinib+ACA (p<0.001), the imatinib+astaxanthin (p<0.001) and the imatinib+astaxanthin+ACA (p<0.001) than in the imatinib. Also, the ROS production was markedly lower in the imatinib+astaxanthin+ACA when compared to the imatinib+astaxanthin (p<0.001).

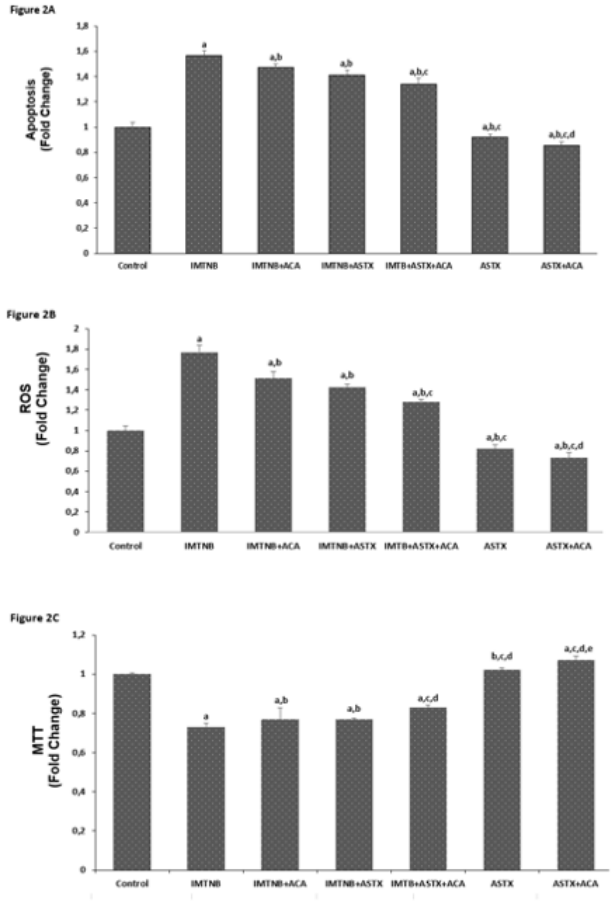

Figure 2A / 2B / 2C. The effect of Imatinib (50 µM, 24 hrs) and Astaxantin (40 µM, 12 hrs) on Apoptosis (A), ROS (B), and MTT (C) levels in the Cardiomyocyte cells. Cardiomyocyte are stimulated by Cumene hydroperoxide (CMPx 0.1 mM for 10 min) but they were inhibited by Antranilic Acid (0.04 mM, 30 min) (mean ± SD and n=10). 2A/2B : ap<0.001 vs control, bp<0.001 vs IMTNB, cp<0.001 vs IMTNB+ASTX and dp<0.001 vs ASTX .2C: ap<0.001 vs control, bp<0.05 and cp<0.001 vs IMTNB, dp<0.001 vs IMTNB+ASTX and ep<0.001 vs ASTX

MTT (cell viability) values in the groups are shown in figure 2C. MTT values were lower in the imatinib than in the control (p<0.001). The values were significantly higher in the imatinib+ACA (p<0.05), the imatinib+astaxanthin+ACA (p<0.001) and the imatinib+astaxanthin (p<0.05) than in the imatinib. In addition, MTT values were higher in the imatinib+astaxanthin+ACA when compared to the imatinib+astaxanthin (p<0.001).

Effects of imatinib and astaxanthin on intracellular ROS production in cardiomyocytes

Effects of imatinib and astaxanthin on caspase 3, caspase 9 activities, mitochondrial depolarization levels in cardiomyocytes

Mitochondrial membrane depolarization levels, caspase-3 and -9 activities of groups are shown in figure 3A,3B,3C respectively. It has been shown that caspase 3 and 9 activities have an important role in the mitochondrial apoptotic pathways. Also, they are associated with mitochondrial cytochrome c release during the apoptotic cascade.

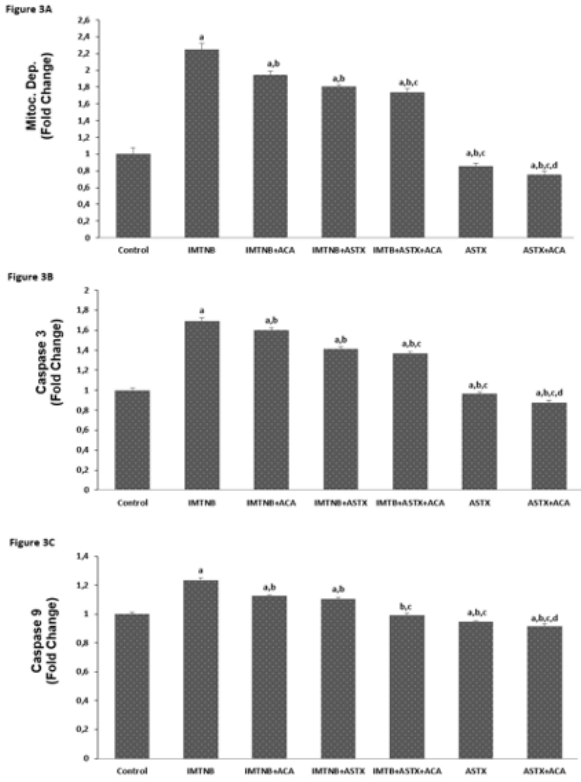

Figure 3A /3B / 3C. The effect of Imatinib (50 µM, 24 hrs) and Astaxantin (40 µM, 12 hrs) on Mitochondrial Depolarization (A), Caspase 3 (B) and Caspase 9 (C) levels in the Cardiomyocyte cells. Cardiomyocyte are stimulated by Cumene hydroperoxide (CMPx 0.1 mM for 10 min) but they were inhibited by Antranilic Acid (ACA 0.04 mM for 30 min) (mean ± SD and n=10). 3A: ap<0.001 vs control, bp<0.001 vs IMTNB, cp<0.05 vs IMTNB+ASTX and dp<0.001 vs ASTX. 3B/3C: ap<0.001 vs control, bp<0.001 vs IMTNB, cp<0.001 vs IMTNB+ASTX and dp<0.001 vs ASTX .

The mitochondrial depolarization, caspase 3, caspase 9 activities values were higher in the imatinib group than in the control ($p<0.001$). The mitochondrial depolarization, caspase 3, caspase 9 values were significantly lower in the imatinib+ACA ($p<0.001$), the imatinib+astaxanthin ($p<0.001$) and the imatinib+astaxanthin+ACA ($p<0.001$) than in the imatinib. Also, the mitochondrial depolarization, caspase 3, caspase 9 values production was markedly lower in the imatinib+astaxanthin+ACA when compared to the imatinib+astaxanthin (Mit. dep. $p<0.05$; caspases $p<0.001$).

DISCUSSION

In our study, we observed that imatinib increased oxidative stress, cytosolic calcium levels and apoptosis in cardiomyocytes. Imatinib mesylate is one of the first tyrosine kinase inhibitor and represents a revolution in the management of patients with chronic myeloid leukemia [13]. Cardiotoxic effect of imatinib was reported first in 2006 by Kerkala et al. Authors collected clinical data from patients who developed left ventricular dysfunction after few months of imatinib therapy. They reported that imatinib had detrimental effects on cardiomyocytes in culture and ultra-structured evaluations revealed mitochondrial dysfunction and prominent membrane whorls in myocytes [3]. Existing data strongly suggests that alterations in endoplasmic reticulum stress pathways leading to mitochondrial function impairment, has an essential role in the cause of imatinib -induced cardiotoxicity [5]. In a study by Perik et al., it was been suggested that only patients with a history of cardiac disease should have cardiac monitorization during imatinib therapy [14]. However, in a clinical trial for imatinib mesylate, Cohen et al. reported left ventricular dysfunction and congestive heart failure in patients without a prior history [15]. These studies revive questions regarding the potential cardiotoxic effects of imatinib mesylate therapy. A more recent report has noted that imatinib mesylate related adverse effects are associated with oxidative stress and mitochondrial dysfunction [16]. Furthermore, previous study showed that imatinib could target cardiomyocytes and mitochondrial dysfunction and cell death could be aggravated by the presence of oxidative stress [17]. Reactive oxygen species and oxidative stress parameters seem to play an important role in chemotherapy-induced cardiotoxicity, leading to cardiac dysfunction [18]. Mitochondria has an important role in cell survival as well as apoptosis. Also, it has been known that increased cytosolic calcium levels stimulate respiratory chain activity and oxidative stress which lead to release proapoptotic factors [19]. It remains controversial whether left ventricular dysfunction with imatinib mesylate is associated with myocyte death or myocyte dysfunction. Barr et al. has suggested that imatinib can alter intracellular calcium levels and has a potential to cause cardiomyopathy resulting from cell death [20]. Calcium plays a crucial role in cardiomyocyte homeostasis and survival. Transient receptor potential (TRP) family are one of the important plasma membrane transporters of calcium ions. Transient receptor potential channels influence cell death rates and affect different pathologies including cardiovascular, neurological, metabolic or neoplastic disorders [21]. TRP melastatin 2 (TRPM2) is the second member of the TRP melastatin subfamily and is expressed in many cell types including brain, heart and endothelial cells [22]. It has been demonstrated that TRPM2 channels are involved in several physiological processes, such as oxidative stress and apoptosis [23]. Wang et al. reported that oxidative stress activated TRPM2 channels and then by positive feedback, it further induces intracellular ROS production and causes loss of mitochondrial membrane potential [24]. In another study, it has been shown that calcium entry via TRPM2 is important in maintaining mitochondrial function and reducing oxidative stress in cardiomyocytes [25]. Also, the results of the previous studies showed that, TRPM2 channels are mainly associated with calcium overload, mitochondrial dysfunction and apoptosis signaling pathway [26].

In our study we observed that TRPM2 channels are present in cardiomyocytes and they are stimulated by cumene hydroperoxide, whereas blocked by antranilic acid respectively. The results of the present study demonstrated that imatinib mesylate increased oxidative stress, calcium entry and apoptosis in cardiomyocytes. In addition, we observed that caspase 3, caspase 9 and intracellular ROS production values were decreased by astaxanthin administration in

cardiomyocytes through modulation of TRPM2 channels.

In the light of the data from previous studies, it has been known that left ventricular dysfunction and many other conditions that predispose heart failure are associated with oxidative stress [27]. Several studies have demonstrated beneficial effects of a therapy with antioxidant agents against endothelial dysfunction, ischemia-reperfusion injury or cardiotoxic agent-induced myocardial damage [28,29]. Astaxanthin is a xantophyll carotenoid that is found in a variety of living organisms especially in the marine environment. It has antioxidant and anti-inflammatory effects. In a review about astaxanthin in cardiovascular disease, it has been reported that biomarkers of oxidative stress and inflammation are decreased by astaxanthin supplementation [30]. Chemotherapy-induced cardiotoxicity is a life-threatening complication which limits the clinical use of chemotherapeutic agents. Also understanding the molecular mechanisms of chemotherapy induced cardiotoxicity is necessary to improve effective preventive strategies. To the best of our knowledge, there is no study that examines the effect of using a combination of imatinib mesylate and astaxanthin on apoptosis, oxidative stress and calcium influx through TRPM2 channels in cardiomyocytes. The present study demonstrated that astaxanthin modulates imatinib-induced oxidative stress and apoptosis through TRPM2 channels. Also, we observed that astaxanthin suppressed mitochondrial depolarization levels and had protective effect on the apoptosis as indicated by caspase 3 and caspase 9 values in myocytes.

In conclusion, understanding the pathophysiological mechanism, as well as an increased awareness of chemotherapy-induced cardiotoxicity, could improve clinical care of cancer patients. Astaxanthin can be a useful preventive agent and TRPM2 channels can be potential therapeutic targets in patients with imatinib-induced cardiotoxicity.

Study limitations

In the present study, we did not evaluate concentration response to distinguish effects of different toxic levels of imatinib on the molecular mechanism studied. Additionally, we were not able to perform an electrophysiological study and evaluate if imatinib and astaxanthin administrations also change the expression of TRPM2 channels in cardiomyocytes.

Conflict of Interest: No conflict of interest was declared by the authors

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RESEARCH ARTICLE

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Can appendix bending angle be an additional finding to detect acute appendicitis on MDCT?

Akut appendisitin ÇKBT tanısında appendiks bükülme açısı ek bir tanısal bulgu olabilir mi?

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ABSTRACT

Aim: Computed tomography (CT) is frequently used as an imaging modality in the evaluation of acute appendicitis. The most frequently used measurement to detect acute appendicitis is maximal outer diameter of appendix on CT and several studies have reported other CT findings of acute appendicitis, such as thickness of the appendiceal wall, appendiceal wall enhancement, peri-appendiceal free fluid, peri-appendiceal inflammation, peri-appendiceal lymph node, appendicolith and cecal wall thickening. We investigated the value of the appendix bending angle (ABA) as an additional and novel finding in the diagnosis of acute appendicitis.

Methods: This retrospective study was conducted after the local ethics committee's approval. 52 consecutive patients who underwent appendectomies were assigned to the study group. The patients, who underwent abdominopelvic CT for any other reason than acute abdomen, were included as control group.

Results: The mean age of the appendicitis group was 41.9±16.0; male predominance was present (n=32, 61.5%). Peri-appendiceal inflammation was seen in 65.4%, peri-appendiceal lymph node was seen in 73.1% and appendicolith was present in 9.6% of the appendicitis group. The mean ABA was 103.0±15.9 degree in study group and 118.8±23,8 degree in control group respectively (p<0.001). The sensitivity of ABA was calculated as 76.9% and the specificity was 58.3% at 113.15 degree which is the best cut-off point calculated by ROC curve. There was no appendicitis over 142.3 degrees.

Conclusion: ABA can be used as an additional finding which is decreased in acute appendicitis.

Key words: acute, appendicitis, angle, multidetector computed tomography

ÖZ

Amaç: Bilgisayarlı tomografi (BT), akut apandisit tanısında sık kullanılan bir görüntüleme yöntemidir. Akut apandisiti saptamak için BT'de en sık kullanılan ölçüm; apendiks en geniş dış çapının transvers düzlemde ölçümüdür. Birçok çalışma apendiks duvar kalınlığı, apendiks duvar kontrastlanması, peri-apendiküler serbest sıvısı, peri-apendiküler enflamasyon, peri-apendiküler lenf nodu, apendikolit ve çekum duvar kalınlaşması gibi akut apandisitin diğer BT bulgularını bildirilmiştir. Biz çalışmamızda akut apandisit tanısında apendiks bükülme açısının (ABA) tanıya ek ve yeni bir bulgu olarak katkısını araştırdık.

Yöntemler: Bu retrospektif çalışma enstitüden etik kurul onayı alındıktan sonra yapıldı. Çalışma grubuna ardışık tarihlerde apendektomi yapılan 52 hasta alındı. Akut batın dışı nedenlerle abdominal BT çekilen hastalardan da kontrol grubu oluşturuldu.

Bulgular: Akut apandisit grubunun yaş ortalaması 41.9 ± 16.0 idi. Erkek hastalar çoğunluktaydı (n = 32, %61.5). Akut apandisit grubunda peri-apendiküler inflamasyon %65.4, peri-apendiküler lenf nodu %73.1 ve apendikolit %9.6 oranlarında pozitif bulundu. Ortalama ABA, çalışma grubunda 103.0 ± 15.9 derece, kontrol grubunda ise 118.8 ± 23.8 derece idi (p <0.001). ABA'nın duyarlılığı % 76.9 olarak hesaplandı ve özgüllüğü ROC eğrisi ile hesaplanan en iyi kesme noktası olan 113.15 derecede % 58.3 idi. 142.3 derecenin üzerinde akut apandisit saptanmadı.

Sonuç: ABA'nın azalmış olması akut apendisit tanısında ek bir bulgu olarak kullanılabilir.

Anahtar Kelimeler: akut, apendisit, açı, çok kesitli bilgisayarlı tomografi

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INTRODUCTION

Acute appendicitis is the most common cause of acute abdomen that requires surgery, with an estimated lifelong risk of 8.6% in men and 6.7% in women [1]. It is often regarded as a disease of the young population with a peak incidence in the second and third decades of life [1]. Appendectomy is generally accepted as a first-line treatment for non-complicated acute appendicitis [2]. Plain radiography, ultrasonography and computed tomography and even magnetic resonance imaging have all been used in diagnosis.

Computed tomography (CT) has been frequently used as an imaging modality in the evaluation of acute appendicitis and has improved the diagnostic ability, leading to a significant reduction in the number of negative appendectomies [3]. The use of CT has led not only to a substantial decrease in the rate of unnecessary appendectomies, but also to a concomitant decrease in the perforation rate [4]. With reported sensitivities of up to 83–99% and specificities of 92–99%, CT plays a major role in the clinical decision-making process in acute appendicitis and is considered as a first-line and rapid imaging modality in the diagnostic workup for suspected acute appendicitis [5,6]. A normal appendix can be identified in 73-82% of patients with thin-section axial CT abdomen; also, it can be increased to 93% by using 16-slice CT and multiplanar reformation images [7]. Additionally, using a 64-slice multi-detector CT (MDCT) with coronal reformations can increase the identification rate up to 98.5% [8].

Several studies have reported CT findings of acute appendicitis [9-12]. However, there are no reported conflicting results about the appendix bending angle (ABA) and no available data as to whether a cut-off point influence ABA in adults, with or without acute appendicitis. In this study we hypothesized that as an empty small intestinal segment; the appendix gets rigid and bends towards the cecum at an acute angle. However, a non-inflamed appendix has a relax lining and positioned across the cecum at an obtuse angle. Establishing normal and abnormal parameters for ABA is important because in subtle cases this angle can be helpful for the correct diagnosis. Furthermore, we believe ABA measures can be a precursor for an inflamed appendix and thus, in this study, we investigated signs of inflamed and noninflamed appendix on MDCT and reference values for ABA in symptomatic and asymptomatic adults, using reformatted CT imaging.

MATERIAL AND METHODS

This retrospective study was approved by the local ethics committee of Diskapi Yildirim Beyazit Training and Research Hospital (approval date: 16.01.2017, no: 34/26). From January 2016 to October 2016, 63 patients who underwent appendectomy (laparoscopic or open) for suspected acute appendicitis and diagnosed as acute appendicitis surgically, were included the study. Of these, 11 patients were excluded for the following reasons: preoperative CT was not performed (n=4), CT scanning without using intravenous contrast media (2), complicated appendicitis (abscess, phlegmon, and focal defect in the appendiceal wall, n=3) and poor CT image quality (n=2). In total, 52 consecutive patients were included in the study group. For the aim of the control group, patients who underwent contrast enhanced abdominopelvic CT for causes other than acute abdomen were enrolled consecutively between the same dates of the study group.

MDCT imaging

MDCT examinations were performed with a 128-detector row CT machine (GE Optima 660 SE 64 Detector 128-slice CT, General Electric Medical Systems, Milwaukee, WI). CT protocols were based on the following: effective level of 140–175 mAs, 120 kVp, 0.625 collimation, 5-mm thickness reconstruction at 5-mm intervals, 0.5-second rotation time. 100–120ml iodinated contrast medium was injected via the antecubital vein at a rate of 3mL/second, with a delay of 60 seconds between contrast administration and data acquisition given at a rate of 3-3.5 mL/s. We did not use enteric contrast material, as the need for it is questionable according to recent studies [13,14]. Images were acquired from the dome of the diaphragm through the pubic symphysis. Both transverse, sagittal and coronal reconstruction images were obtained. Soft tissue kernel was used and the reconstruction increment was 0.625-mm.

Image analyses: Two abdominal radiologists who were blinded to the histopathologic and surgical findings evaluated in consensus all images retrospectively. They evaluated the following features: transverse diameter of the appendix, thickness of the appendiceal wall, appendiceal wall enhancement, peri appendiceal free fluid, peri appendiceal inflammation, existence of peri-appendiceal lymph node, appendicolith and cecal wall thickening [15]. The transverse diameter of the appendix and thickness of the appendiceal wall were measured at maximal short-axis diameter and maximal wall thickness of the inflamed appendix, respectively. Appendiceal wall enhancement was compared to that of the cecal wall and divided into two groups: increased or un-increased [15]. Images were evaluated for ABA on coronal reformat images. ABA was defined as the wide angle between long axis of cecum and long axis of proximal appendix. Figure 1 showed ABA measurement on normal and inflamed appendix. Each observer measured ABA on the same day in separated computers to assess interobserver variation. Additionally, to detect intraobserver variations, observers measured the ABA for the second time in two-week interval. Observers were blinded to their previous measurements. The final measurement of continuous variables were calculated as follows: The mean of the two measurement of the first observer was added to the second observers result and divided into two again [Result= 0.5 x ((First look of Observer1 + Second look of Observer1) x 0.5 + Observer2)].

Statistical Analyses:

The statistical calculations were performed by "IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.". The continuous variables were expressed as mean±sd, categorical data was expressed as n (%). The normal distribution was determined by histogram and Kolmogorov-Smirnov test. To compare the mean of two groups that were normally distributed, Student's t Test was used. The categorical parameters were compared by Chi Square test. ROC analysis was used to determine the cut-off points; sensitivity and specificity values were calculated. Correlation of the same raters two judgments was calculated by Pearson Correlation Coefficient and correlation between two radiologists by Kendall's Coefficient of Concordance (the mean value of the first observer compared with the second observer's measurement). All tests was applied as two tailed and the statistical significance level was $p<0.05$.

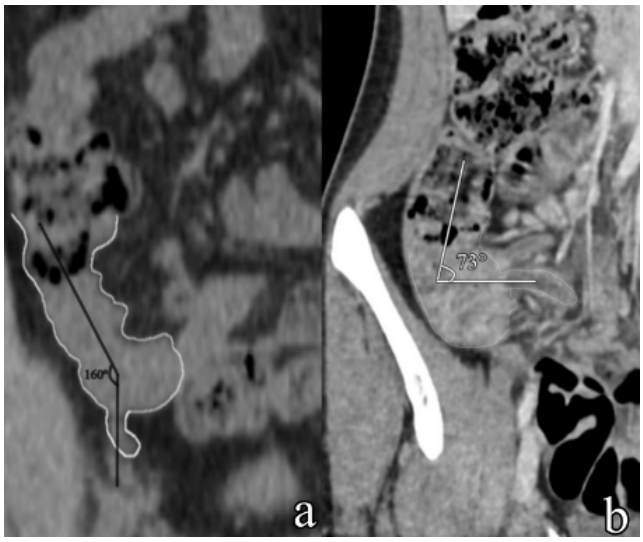

Figure 1: (a) Normal appendix. Appendix bending angle measurement technique in coronal plane (the angle between the vertical line of cecum and appendix) (b) Acute appendicitis. Measurement of ABA in coronal plane.

RESULTS

The mean age of the appendicitis group was 41.9±16.0 (range 18-77) versus the control group was 45.4±15.2 (range 18-80). Male predominance was present in the appendicitis group (n=32, 61.5%) whereas female dominance was present in the control group (n=27, 56.3%).

The frequency of peri appendiceal inflammation, peri appendiceal lymph node, appendicolith, as well as the means of appendix wall thickness, cecum wall thickness, appendix diameter and ABA and comparison of appendicitis and control group, were given in Table 1.

Peri appendiceal inflammation was present in 65.4% of the appendicitis group but also in 4.2% of the control group. It is more frequently observed in appendicitis, but the sensitivity was 65.38% and the specificity was 95.83%; positive predictive value (PPV) was 94.44% (81.19% - 98.53%), negative predictive value (NPV) was 71.88% (63.65% to 78.86%).

Peri appendiceal lymph node was present in 73.1% of the appendicitis group but also in 14.6% of the control group. It was more frequently

observed in appendicitis, but the sensitivity was 73.08% and the specificity was 85.42%; positive predictive value (PPV) was 84.44% (72.86% - 91.65%), negative predictive value (NPV) was 74.55% (64.83% to 82.31%).

Table 1. Frequency of appendicitis findings and measurements of appendix in appendicitis and control group

	Appendicitis Group		Control Group		P
	n	%	n	%	
Periappendiceal inflammation	34	65,40%	2	4,20%	<0,001
Periappendiceal lymph node	38	73,10%	7	14,60%	<0,001
Appendicolith	5	9,60%	-	-	<0,028
	mean±sd	(min-max)		(min-max)	
Bending angle (degree)	103,0± 15,9	(69,5-142,3)	118,8± 23,8	(75,8-160,0)	<0.001
Appendix diameter (mm)	10,14± 1,53	(5,0-13,0)	4,66± 0,46	(3,5-5,3)	<0.001
Appendix wall thickness (mm)	3,87± 0,68	(2,23-5,43)	2,42± 0,29	(1,88-3,18)	<0.001
Cecum wall thickness (mm)	4,35± 0,84	(2,48-6,38)	2,65± 0,40	(2,00-3,90)	<0.001

Appendicolith was present in 9.6% of the appendicitis group but in none of the control group. It was observed in appendicitis, but the sensitivity was 96.2% and the specificity was 100%; positive predictive value (PPV) was 100%, negative predictive value (NPV) was 50.53% (48.31% to 52.74%).

The mean of appendix wall thickness, cecum wall thickness and appendix diameter were greater in appendicitis group as expressed in Table 2, ROC curve and best cut-off points of these measurements were given in Figure 2. The mean ABA was 103.0±15.9 in acute appendicitis group and 118.8±23.8 in control group, respectively. The ROC curve of appendix bending angle was showed in Figure 3. Area under curve in ROC analysis was 0.695 (0.588-0.801; 95%CI). Two cut-off points with sensitivity and specificity values were given in Table 2. According to ROC curve the best cut-off of point was 113.15 degrees; at this point the sensitivity was 76.9% and the specificity was 58.3% (PPV 66.67% and NPV 70.00%). When we got the cut-off of point as 110 degrees the sensitivity would be 69.2% and the specificity would be 60.4% (PPV 65.5% and NPV 63.6%). As we saw in Table 1, there were no appendicitis cases over the bending angle 142.3 degrees.

Table 2. AUC and cut-off points determined by ROC curve and sensitivity and specificity of these points

	AUC* of ROC** Curve (Upper-Lower Bound with 95% CI***)	Cut-off Point	Sensitivity (%)	Specificity (%)
Appendix diameter (mm)	0,994	6,3	98,1	100,0
	(0,981 - 1,000)			
Appendix wall thickness (mm)	0,982	3,08	96,2	97,9
	(0,953 - 1,000)	3,20	88,5	100,0
Cecum wall thickness (mm)	0,970 (0,937 - 1,000)	3,27	96,2	95,8
		3,94	63,5	100,0
Bending angle (degrees)	0,695	113,15	76.9	58,3
	(0,588-0,801)	110,00	69,2	60,4

*AUC: Area under curve, **ROC: Receiver operating characteristic, ***CI: Confidence interval

There was a high correlation between two measurements of the same observer (p<0.001, r=0.884). However, interobserver reliability was lower compared to intraobserver rates (p=<0.001, r=0.262).

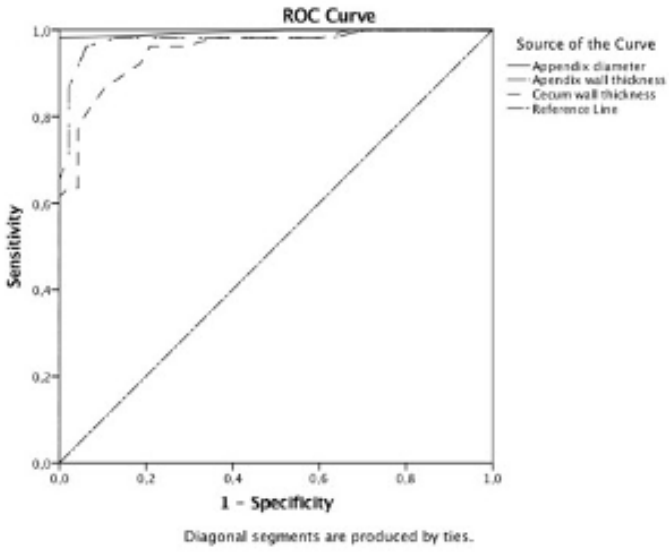

Figure 2. ROC curve of appendix wall thickness, cecum wall thickness and appendix diameter

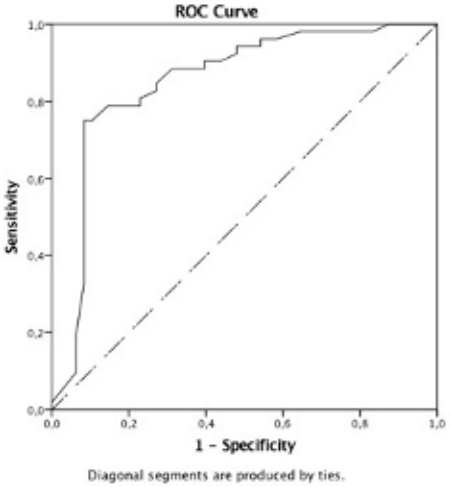

Figure 3. ROC curve of appendix bending angle

DISCUSSION

Diagnosis of acute appendicitis can be determined by observing the increased diameter of the appendix, the presence of appendicolith, peri appendiceal inflammation and free fluid on the CT [15]. Except those on MDCT, as in our hypothesis, ABA can be measured as an additional and a new solution in the diagnosis of acute appendicitis.

Firstly, if we evaluate at the classic CT findings of appendicitis, we find in our study results that are similar to previous studies, such as an increased diameter and wall thickness of the appendix, increased cecum wall thickness, the presence of appendicolith, peri appendiceal inflammation and lymph node all highly indicate the presence of appendicitis. The inflamed appendix is distended with a diameter measuring between 6-40 mm and a wall thickness of 1–3mm [16]. Our study revealed that the mean appendix diameter was 10,14±1,53 mm in acute appendicitis group. Besides over 6.3 mm of appendix diameter the sensitivity was 98.1% and specificity was 100%. Several studies report that the diameters of normal appendices do not exceed 6 mm [17,18], while others reported diameters greater than 6 mm even up to 10mm [19,20]. We did not detect an appendix diameter over 6mm in our control group. Additionally, we measured the mean wall thickness of appendix as 3.87±0.68 (range 2.23-5.43) in the acute appendicitis group and 2.42±0.29 (range 1.88-3.18) in the control group.

Appendicoliths is a rare finding of the acute appendicitis but it is associated with severe appendicitis, appendiceal perforation, recurrent appendicitis after conservative therapy or failure of antibiotic therapy [21]. The presence of appendicoliths and the location of an appendicolith at the root of the appendix were significantly associated with gangrenous appendicitis [22]. As described, appendicolith is very rare, it was observed in 9.6% of the appendicitis group and not at all in the control group. Another study has reported the frequency of appendicolith as 33.3% in acute appendicitis and never observed in their control group [23]. Peri appendiceal inflammation is one of the positive findings of acute appendicitis [24]. Tatar et al. reported that in the patients with normal appendix mild to moderate peri appendiceal inflammation frequency was 12.8% and severe peri appendiceal inflammation was 3.8%; in the patients with acute appendicitis, mild to moderate peri appendiceal inflammation was observed in 30.8% of the patients and severe peri appendiceal inflammation was observed in 48.7% of the patients [23]. Our study results were similar with the previous literature: we observed peri appendiceal inflammation in 73.1% of the appendicitis group and 14.6% of the control group.

In this study, we hypothesized that ABA decreases in cases of acute appendicitis and we could not find a similar study about this angle and its association with acute appendicitis. The mean ABA in acute appendicitis was significantly lower than in the control group, which supports our hypothesis that when we come up with as an empty small intestinal segment, an inflamed appendix forms an acute angle throughout the cecum. The sensitivity of ABA was calculated as 76.9% and the specificity was 58.3% at the point of 113.15 degrees which is the best cut-off point and the specificity was 59.6% at the point of 110 degrees. There was no appendicitis case over the bending angle of 142.3 degrees. With these results, we determined that ABA can be used as a novel and additional finding which can exclude acute appendicitis over 142.5 degrees and which additionally, has acceptable sensitivity and specificity values at 113.15 or 110 degrees. Furthermore, we explained the predictive

value of ABA in acute appendicitis, which is significantly high to be used as a diagnostic tool.

Having been designed retrospectively, a limitation of this study is the low number of patients included and lack of knowledge whether the patients were in the early phases of inflammation or not. Our results should be further validated with a higher number of patients and with the addition of follow-up periods, in order to build more solid recommendations after a standardized explanation of measurement method to the observers.

In conclusion, CT is an accurate imaging modality for the diagnosis of acute appendicitis. The main purpose of our study was to determine the diagnostic value of ABA, which can be used as a new diagnostic finding that was found to be lower in patients with acute appendicitis. There were no cases with acute appendicitis over 142.3 degrees. But further prospective studies with patients that are conservatively followed will provide better results that can be used in clinical practice.

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RESEARCH ARTICLE

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Evaluation of Tuberous Sclerosis Complex Patients

Tüberoskleroz kompleksi tanılı hastaların değerlendirilmesi

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ABSTRACT

Aim: Tuberous sclerosis complex (TSC) is a multisystem genetic, autosomal-dominant disorder predisposing to multiple organ manifestations. The aim of this study is to determine TSC the frequency of findings including diagnostic and non-diagnostic criteria.

Patients and Metod: Thirty-five patients diagnosed with tuberous sclerosis complex were examined retrospectively. The diagnosis of the patients were evaluated according to the diagnostic criteria of TSC that were updated in 2012. As non-diagnostic criteria, we reviewed epilepsy, drug-resistant epilepsy, electroencephalography (EEG) types (focal, diffuse-multifocal and hypsarrhythmia) and TAND (TSC-associated neuropsychiatric disorders) (intellectual disability and/or autism and learning disability).

Results: Twenty-one cases (60%) presented with seizures, 9 cases (26%) with hypopigmented patches and 5 cases (14%) with cardiac rhabdomyomas. The most common finding with brain magnetic resonance imaging (MRI) was cortical tubers (85%). EEG examinations revealed diffuse and multifocal epileptic disorder in 5 (24%), focal epileptic disorder in 8 (38%), and hypsarrhythmia in 8 (38%) patients. 38% of the patients with epilepsy were diagnosed with refractory epilepsy. Severe intellectual disability and / or autism were detected in 11 (32%) patients. The number of patients with renal angiomyolipoma (p:0.001) were significantly higher in drug resistant epilepsy patients and also TSC-associated neuropsychiatric disorders (TAND) (p:0.001) rate was significantly higher in epilepsy patients.

Conclusion: The disease should be followed with a multidisciplinary approach. Although not included in the diagnostic criteria, it should be kept in mind that epilepsy, intellectual disability and neuropsychiatric disorders frequently accompany.

Keywords:Tuberoussclerosis Complex, Epilepsy, Intellectual Disability

ÖZ

Amaç: Tüberoskleroz kompleksi (TSK) vücutta birçok organın tutulumu ile karakterize, otozomal dominant kalıtım gösteren genetik bir rahatsızlıktır. Bu çalışmada TSK tanı kriterlerinin ve tanı kriterleri dışındaki bulguların sıklığını belirlemek amaçlanmıştır.

Hastalar ve Yöntemler: TSK tanılı 35 hastanın verileri geriye dönük olarak incelendi. Hastaların tanısı, 2012 yılında güncellenen TSK'nin tanı kriterlerine göre değerlendirildi. Tanısal olmayan kriterler olarak; epilepsi, ilaca dirençli epilepsi, elektroensefalografi (EEG) tiplerini (fokal, diffüz-multifokal ve hiperaritmi) ve TAND'ı (TSC ile ilişkili nöropsikiyatrik bozukluklar) (zihinsel yetersizlik ve / veya otizm ve öğrenme yetersizliği) inceledik.

Bulgular: Yirmi bir hasta (%60) nöbet geçirme, 9 hasta (%26) hipopigmente lekelenmeler ve 5 hasta (%14) kardiyak rabdomiyomlar nedeniyle başvurmuştu. Beyin magnetik rezonans görüntüleme (MRG) ile en sık saptanan bulgu kortikal tüberlerdi (%85). Nöbet geçiren hastaların EEG incelemelerinde 5'inde (%24) yaygın ve multifokal epileptik bozukluk, 8'inde (%38) fokal epileptik bozukluk ve 8'inde (%38) hipsaritmi paterni saptandı. Epilepsi tanısı ile izlenen olguların %38'i dirençli epilepsi tanısına sahipti. Ağır derecede entelektüel yetersizlik ve/veya otizm 11 (%32) hastada saptandı. Dirençli epilepsi grubunda böbrek anjiomiyolipomaları olan hasta sayısı anlamlı olarak fazlaydı (p:0.001) ve aynı zamanda tüberoskleroz ile ilişkili nöropsikiyatrik bozuklukların oranı anlamlı olarak epilepsi grubunda yüksekti (p:0.001).

Sonuç: Hastalığın multidisipliner bir yaklaşım ile takip edilmesi gerekmektedir. Tanı kriterlerinde yer almasa da epilepsi, entelektüel yetersizlik ve nöropsikiyatrik bozuklukların sık eşlik ettiği akılda tutulmalıdır.

Anahtar Sözcükler:Tüberoskleroz Kompleksi, Epilepsi, Entelektüel Yetersizlik

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INTRODUCTION

Tuberosclerosis complex (TSC) is a single gene disorder with an autosomal dominant inheritance pattern and a frequency of 1/6000 – 1/10000 live births [1,2]. It is caused by a mutation in the TSC1 and TSC2 genes which impairs the function of hamartin and tuberin complex, and clinical findings appear because of the inhibitory effect of rapamycin on the mammalian target (mTOR) signaling pathway [2,3]. Involvement of different organs are observed in different age groups. It is known that brain and heart findings can be seen from the prenatal period, skin and kidney findings are seen during childhood and pulmonary findings frequently present in adulthood [4,5].

Central nervous system (CNS) involvement occurs in more than 90% of patients. This involvement is observed as cortical-subcortical tubers, subependymal nodules, giant cell astrocytoma (SEGA) and white matter radial migration lines. Epilepsy, mental retardation, autism and additional neuropsychiatric disorders occur in these patients as a result of CNS involvement [6]. All types of epileptic seizures can be observed in TSC and these seizures are generally resistant to antiepileptic treatment [7].

The diagnostic criteria of the disease were updated in 2012 by the International Tuber Sclerosis Complex Consensus Group [4,5]. In this study, we retrospectively evaluated data of the patients with TSC disease and analysis the frequency of signs and symptoms that were both included and non-included in diagnostic criteria.

PATIENTS and METHODS

A total of 35 patients with TSC were recruited at the Pediatric Neurology Department from January 2013 to December 2017. Clinical, laboratory and imaging findings of the patients were evaluated retrospectively. The study included patients aged 1-17 years who were followed up for a diagnosis of TSC for at least 1 year. The diagnosis of the patients were evaluated according to the diagnostic criteria of TSC that were updated in 2012 by the International Tuber Sclerosis Complex Consensus Group [4,5]. As non-diagnostic criteria, we reviewed epilepsy, drug-resistant epilepsy, electroencephalography (EEG) types (focal, diffuse-multifocal and hypsarrhythmia) and TAND (TSC-associated neuropsychiatric disorders) (intellectual disability and/or autism and learning disability).

This study was approved by the ethics review committee of the Akdeniz University Clinical Research with 06.26.2019 date number of 607 decision in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

Statistical Analysis: All analyses were performed on SPSS v17.00 (SPSS Inc., Chicago, IL, USA). Descriptive data of the patients were analyzed. In respect to normality of distribution, data given as mean ± standard deviation or median (minimum - maximum) for continuous variables, and frequency (percentage) for categorical variables. Categorical comparisons between groups were performed by the Chi-Square and p values lower than 0.05 were considered as statistically significant.

RESULTS

We included 35 patients (51.4% male) into our study, median age was 1.9 years (range from 2 months-14 years). Twenty-one patients (60%) presented with seizures, 9 patients (26%) with hypopigmented spots and 5 patients (14%) presented with cardiac rhabdomyomas (2 of these had been diagnosed in the intrauterine period). Ten (28%) patients had a family history of TSC. Hypomelanotic macules on the skin were present in all patients except for one patient. Cortical tubers (n = 30, 85%) and subependymal nodules (n = 29, 83%) were the most common findings on brain magnetic resonance imaging (MRI) (Figure 1 and 2).

In addition to CNS and skin findings, 8 patients (23%) had rhabdomyoma in the heart, 13 patients (37%) had angiomyolipoma in the kidney and 1 patient (3%) had eye involvement (Table 1). Electroencephalography (EEG) examinations of the patients with seizures revealed diffuse and multifocal epileptic disorder in 5 (24%), focal epileptic disorder in 8 (38%), and hypsarrhythmia pattern in 8 (38%) patients. Seven (33%) patients were receiving treatment with a single antiepileptic drug, 6 (29%) patients were using two, and 8 (38%)

patients were using multiple (>2) antiepileptic drugs. Twenty-two (62.8%) patients had various degrees of intellectual disability and/or autism and learning disability (Table 2). Categorical comparisons between diagnostic and non-diagnostic criteria groups were given in Table 3. The number of patients with renal angiomyolipoma (p:0.001) was significantly higher in drug-resistant epilepsy patients than non-drug resistant epilepsy patients (50% vs 9.5%) and also TSC-associated neuropsychiatric disorders (TAND) (p:0.001) rate was significantly higher in epilepsy patients than non epileptic patients (85.3% vs 28.5%).

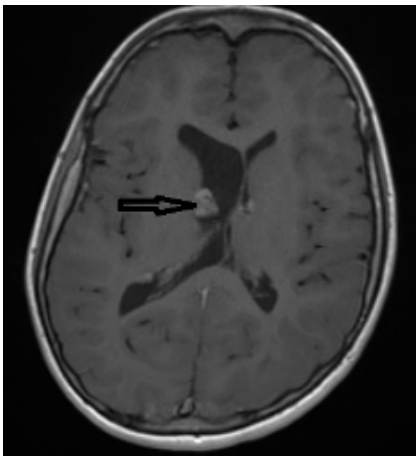

Figure 1. Axial FLAIR MRI shows subependymal nodule along the lateral wall of the lateral ventricle (black arrow).

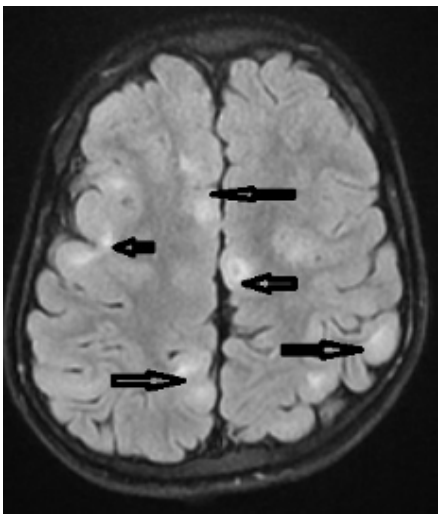

Figure 2. Axial FLAIR MRI shows multiple cortical tubers (black arrow).

DISCUSSION

In this study, we found that hypomelanotic macule was the most commonly seen diagnostic criteria. Also among the symptoms which were not included in diagnostic criteria, the most commonly seen one was learning and intellectual disability. Tuberosclerosis complex is a disease with different symptoms and signs depending on the age of onset and the severity of the disease [8]. In this study, the most common examination finding was hypomelanotic macula (97%). In the literature, hypomelanotic macular incidence in the childhood was reported as 61–97.2% [9,10]. In our study, other common skin findings were facial angiofibroma (34%) and shagreen patch (6%).

Table 1. Evaluation of 35 TSC patients in terms of diagnostic criteria

Criteria	Number of patients (%)
Hypomelanotic macules	34 (%97)
Shagreen patch	2 (%6)
Multiple retinal hamartomas	1 (%3)
Facial angiofibromas	12 (%34)
Cortical tuber	30 (%85)
Subependymal nodules	29 (%83)
Subependymal giant cell astrocytoma (SEGA)	4 (%11)
Cardiac rhabdomyoma	8 (%23)
Renal angiomyolipoma	13 (%37)
Nonrenal hamartomas	7 (%20)
Multiple renal cysts	9 (%25)

Table 2. Evaluation non-diagnostic clinical features of 35 TSC patients

Criteria	Number of patients (%)
Epilepsy	21 (%60)
Learning disability	6 (%22)
Mild type intellectual disability	5 (%14)
Severe type intellectual disability and/or autism	11 (%32)

In this study, 60% of patients had epilepsy diagnosis. This rate ranges between 72-85% in the literature, and was lower in our study [11]. Different types of seizures may occur in patients with TSC. In this study, infantile spasm was present in 38% of patients, and this rate was reported to range from 32.7% to 40% in the literature [12]. In the current study, focal seizures were seen in 38% of the patients, while multifocal seizures were seen in 24%. Similarly, the literature reports that early onset seizures are frequently focal seizures and infantile spasms [13]. Epileptic seizures are also common in TSC, but are not included in the major diagnostic criteria of the disease. There is no clear consensus on the course of epileptic seizures, but they are known to be resistant to treatment

Table 3. Comparison of diagnostic and non-diagnostic criteria in TSC patients

	Epilepsy			EEG types				Drug-resistant epilepsy			TAND*		
	Yes (%)	No (%)	p	H. (%)	F. (%)	M.F(%)	p	Yes (%)	No (%)	p	Yes (%)	No (%)	p
Male	57.9	42.1	0.78	27.2	45.6	27.2	0.56	15.8	84.2	0.28	63	37	0.96
Female	62.5	37.5	0.78	50	30	20	0.56	32.1	68.8	028	62.5	37.5	0.96
Hypomelanotic macules	61.8	38.2	0.21	38	38	24	0.82	23.5	76.5	0.58	61.8	38.2	0.43
Facial angiofibromas	58.3	41.7	0.88	42.8	28.6	28.6	0.81	25	75	0.82	50	50	0.25
Shagreen patch	100	0	0.74	38	38	24	0.94	0	100	0.42	50	50	0.69
Multiple retinal hamartomas	0	100	0.21	NA	NA	NA	NA	0	100	0.58	0	100	0.18
Cortical tuber	56.6	43.4	0.32	41.1	35	23.9	0.81	20	80	0.32	63.3	36.7	0.88
Subependymalnodules	65.5	34.5	0.14	42.1	31.5	26.4	0.16	27.5	72.5	0.14	62	38	0.83
SEGA**	50	50	0.66	50	0	50	0.45	25	75	0.91	50	50	0.57
Cardiac rhabdomyoma	62.5	37.5	0.86	60	20	20	0.48	25	75	0.86	50	50	0.39
Renal angiomyolipoma	58.3	41.7	0.88	57.1	28.5	14.4	0.035	50	50	0.001	66.6	33.4	0.73
Multiple renal cysts	77.7	22.3	0.20	43	28.5	28.5	0.81	33.3	66.7	0.38	66.6	33.4	0.78
Nonrenal hamartomas	42.8	57.2	0.30	0	33.4	66.6	0.13	14.2	85.8	0.54	57.1	42.9	0.72

F: Focal, H: Hypsarrhythmia, M.F:Multifocal , NA:Not Available, TAND* (TSC-associated neuropsychiatric disorders), **Subependymal giant cell astrocytoma (SEGA)

[6,13]. In our study, resistant epilepsy was found in 38% of the patients. Intellectual, behavioral and psychosocial disorders reportedly occur in 44-65% of the patients with TSC throughout their lives [14]. Autism spectrum disorders, attention deficit and hyperactivity disorder, various degrees of intellectual disabilities, learning disabilities in different fields (mathematics, reading and writing) or memory and executive dysfunctions may be seen in patients with TSC [5,14]. Our results showed that 46% of the patients had mild to severe intellectual disability and / or autism. In 22% of the patients, learning disabilities were found in different areas despite normal mental development. In 2012, the Tuberosclerosis Neuropsychiatry Panel of the Consensus Group of the International Tuberous Sclerosis Complex Group highlighted this issue and this group of symptoms and conditions were named as 'TSC associated neuropsychiatric disorders' (TAND) [4,5]. We found that neuropsychiatric disease rate was higher in epilepsy group. In another study, they also found this correlation [15]. There has been increasing concern regarding the cumulative neurobiological burden associated with the risk of progressive cognitive impairment and epilepsy. Timing and treatment modalities of epilepsy become important for reducing of TAND [16].

Central nervous system lesions seen in TSC include cortical tubers, white matter heterotopia, subependymal nodules and subependymal giant cell astrocytoma [17]. It has been reported that lesions are often found in the frontal and temporal regions, are seen in 80-95% of patients, and the size and number of lesions are often unrelated to the clinical situation [11-12]. Tubers occur in the intrauterine period and the number of tubers do not change in the postnatal period. Tubers do not develop into neoplasms and become calcified over time [18]. The frequency of subependymal giant cell astrocytoma has been reported to be between 10-20% [11,12]. In our study, cortical tubers were found in 85% of the patients and subependymal nodules in 83%, while subependymal giant cell astrocytoma was detected in 11% of the patients.

The disease may also cause cardiac involvement characterized by cardiac rhabdomyomas. They are usually multiple and have good prognosis [19]. Cardiac rhabdomyomas develop in the intrauterine period during which many patients are diagnosed and referred to pediatric neurology departments in the newborn period with a preliminary diagnosis of TSC [4,5]. Approximately 96% of patients with cardiac rhabdomyoma are diagnosed with TSC [12]. In our study, a total of 5 patients (14%) were diagnosed with cardiac rhabdomyoma, of which 2 were intrauterine.

In our study, renal angiomyolipoma was detected in 37% of the patients. The incidence of renal

lesions in TSC increases with age. The most common lesion is angiomyolipoma and it is frequently detected in multiple and bilateral forms. In our series, the number of renal angiomyolipoma patients were significantly higher in drug-resistant epilepsy patients. This result may be related with TSC2 mutation that causes more severe phenotype of TSC [20,21]. In the literature TSC2 mutations more related with severe phenotype and also renal angiomyolipomas [22]. However our patients were not evaluated for genetic mutations. And also TSC2 gene is in close proximity with the autosomal dominant polycystic kidney disease (PCKD) gene, so PCKD may also be seen in these patients [23]. Renin-dependent hypertension may occur in patients with renal lesions. It is recommended that patients with TSC should undergo abdominal MRI examinations both at diagnosis and after diagnosis at intervals of 1 to 3 years. In addition, arterial blood pressure measurement is required in the follow-up of patients. [4,5].

This study has some limitations. The most important limitation of this study is its retrospective nature. Secondly; the limited number of cases evaluated in the study reduces the reliability of statistical analysis between the groups. In addition, for some of patients our follow up period was short so the patients without epilepsy may develop epilepsy in future. Finally, the diagnoses of the patients in the study were based on clinical and radiological findings, none of them had genetic analysis. However, despite these limitations, we think that it will contribute to the literature because of the evaluation of comorbid conditions not included in the diagnostic criteria of tuberous sclerosis.

CONCLUSION

The results of our study are consistent with the literature. TSC is a multisystemic disease and should be followed with a multidisciplinary approach. Individual differences of the patients should be considered. Although epilepsy, intellectual disability and neuropsychiatric disorders are not included in the TSC diagnostic criteria, they often accompany the disease. It is possible that physical and neuropsychiatric characteristics of the disease may change or emerge over time, therefore regular follow-up and controls are very important.

Congress presentation description: 19. National Pediatric Neurology Congress, poster presentation.

Ethic: This study was approved by the ethics review committee of the Akdeniz University Clinical Research with 06.26.2019 date number of 607 decision in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

Conflict of interest: There is no conflict of interest to declare.

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RESEARCH ARTICLE

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Evaluation of Tp-e interval, Tp-e/QT ratio and Tp-e/QTc ratio in patients with acute pancreatitis

Akut Pankreatitli Hastalarda Tp-e Aralığı, Tp-e/QT Oranı and Tp-e/QTc Oranı'nın Değerlendirilmesi

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ABSTRACT

Aim: Acute pancreatitis may affect cardiovascular system regardless of etiology. Electrocardiographic parameters such as QT interval, corrected QT interval (QTc), Tp-e interval and Tp-e/QT, Tp-e/QTc ratio can be used to evaluate myocardial repolarization. We aimed to investigate the effects of acute pancreatitis on the cardiovascular system and the relationship between ventricular repolarization parameters and the severity of the disease.

Methods: Ventricular repolarization parameters (QT interval, QTc interval, Tp-e/QT, Tp-e/QTc ratio) of the patients who were included in the study and diagnosed with acute pancreatitis were compared with the control group patients. In addition, these parameters and Ranson, APACHE II and amylase values were taken into account in all patients in the pancreatitis group and the relationship between the severity of the disease and cardiac parameters was investigated.

Results: 60 patients (30 acute pancreatitis and 30 control) were examined. Tp-e interval, Tp-e/QT and Tp-e/QTc ratios were significantly higher in the acute pancreatitis group compared to the control. In addition, Tp-e interval, Tp-e/QT and Tp-e/QTc ratios showed a positive correlation with the Ranson and APACHE II scores and Tp-e interval displayed a positive correlation with amylase levels.

Conclusions: Tp-e interval, Tp-e/QT and Tp-e/QTc ratios can be used as a marker for the detection of ventricular arrhythmia risk in acute pancreatitis patients and Tp-e/QT and Tp-e/QTc ratios increased depending on the severity of AP and Tp-e interval increased in parallel with higher levels of amylase. Amylase level alone could be an indicator for increased risk of ventricular arrhythmia in patients with acute pancreatitis.

Keywords: Acute pancreatitis, Electrocardiography, Tp-e interval, Tp-e/QT ratio, Tp-e/QTc ratio

ÖZ

Amaç: Akut pankreatit etyolojisinden bağımsız olarak kardiyovasküler sistemi etkileyebilir. QT aralığı, düzeltilmiş QT aralığı (QTc), Tp-e aralığı ve Tp-e/QT, Tp-e/QTc oranları gibi elektrokardiyografik parametreler myokardiyal repolarizasyonun değerlendirilmesinde kullanılabilir. Çalışmamızda akut pankreatitin kardiyovasküler sistem üzerine olan etkilerini incelemeyi ve hastalığın ağırlığı ile ventriküler repolarizasyon parametreleri arasındaki ilişkiyi ortaya koymayı amaçladık.

Yöntemler: Akut pankreatit tanısı alan hastalarda ventriküler repolarizasyon parametreleri (QT aralığı, QTc aralığı, Tp-e/QT, Tp-e/QTc oranı) kontrol grubu hastaları ile karşılaştırıldı. Ayrıca tüm hastalarda bu parametreler yanında Ranson ve APACHE II skorları ile amilaz değerleri hesaplanarak hastalığın şiddeti ile kardiyak parametreler arasındaki ilişki incelendi.

Bulgular: Çalışmada 60 hasta (30 akut pankreatit ve 30 kontrol) incelendi. Tp-e aralığı, Tp-e/QT ve Tp-e/QTc oranlarının akut pankreatitli hastalarda kontrol grubuna göre istatistiksel olarak anlamlı düzeyde daha yüksek olduğu tespit edildi. Ayrıca Tp-e aralığı, Tp-e/QT ve Tp-e/QTc oranlarının Ranson ve APACHE II skorları ile pozitif korelasyon gösterdiği, Tp-e aralığının amilaz düzeyi ile pozitif korelasyon gösterdiği tespit edildi.

Sonuç: Akut pankreatitli hastalarda Tp-e aralığı, Tp-e/QT ve Tp-e/QTc oranları ventriküler aritmi riskinin tespitinde kullanılabilir ve Tp-e/QT ve Tp-e/QTc oranlarının yükselmesi akut pankreatitin şiddetiyle doğru orantılı olup, Tp-e oranı amilaz düzeyi ile paralel olarak yükselmektedir. Tek başına amilaz düzeyi akut pankreatitli hastalarda ventriküler aritmi riskinin belirleyicisi olarak kullanılabilir.

Anahtar Kelimeler : Akut pankreatit, Elektrokardiyografi, Tp-e aralığı, Tp-e/QT oranı, Tp-e/QTc oranı

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INTRODUCTION

Acute pancreatitis (AP) is a sudden onset pancreatic inflammation that may manifest itself with local and systemic complications and is the 3rd most common gastrointestinal system disease in acute hospital admissions. It has a mortality rate of approximately 5% and is most commonly caused by gallbladder stones [1]. Different scoring methods are used to determine the severity of AP. Among these, the most commonly used pancreatitis-specific scoring system, the Ranson scoring method, is based on the evaluation of some clinical and laboratory parameters at the time of admission and at the 48th hour of hospitalization [2]. Another scoring method, APACHE II, allows the evaluation of the general condition and some physiological functions of the patients starting from the initial admission and grading at 24-hour intervals [3].

In patients with AP, the cardiovascular system may be affected alone or in combination with other systems regardless of etiology. Cardiac rhythm disorders, myocardial contractility disorders, peripheral arterial-venous tone abnormalities are common cardiac manifestations of acute pancreatitis [4]. The pathophysiology of myocardial involvement in AP patients is not fully understood. In addition to systemic inflammatory response, cardiobiliary reflex, toxic effects of pancreatic enzymes on myocardium and coronary artery spasm are physiopathological mechanisms considered in the pathogenesis of cardiac involvement and electrocardiographic changes in acute pancreatitis. Arrhythmias, bradycardia, ST-T wave changes, depolarization and repolarization changes, including intraventricular conduction disturbances, are observed in these patients [5,6].

Parameters such as QT interval, corrected QT interval (QTc), QT dispersion (QTd), and transmural dispersion of repolarization can be used to evaluate myocardial repolarization. Previous studies have shown that the duration between the peak of the T wave and the end point (Tp-e interval) is considered as the total distribution index of repolarization. Similarly, the ratio of Tp-e interval to QT interval (Tp-e/QT) can be used as a reliable marker since it is not affected by heart rate changes in the evaluation of ventricular repolarization. In addition, Tp-e/QT ratio is considered to be a more reliable predictor of ventricular arrhythmogenesis compared to other parameters [7,8].

In this study, we aimed to investigate the effects of acute pancreatitis on the cardiovascular system and the relationship between ventricular repolarization parameters and the severity of the disease.

MATERIALS AND METHODS

The study was performed on patients who were hospitalized and treated for AP between October 2017 and March 2019 in our General Surgery Clinic. Pancreatitis occurred due to biliary stone in all patients. Age, gender, height, weight, body mass index, systolic and diastolic blood pressures, pulse measurements, ejection fraction (EF) values, Ranson and APACHE II scoring values and laboratory parameters used for these scoring methods, along with amylase values measured at initial admission, were recorded. Abdominal ultrasound and abdominal CT scan were performed in all patients. Patients who were found to have cholecystitis at the time of admission and who had cholecystitis and/or pancreatitis attacks 1 month prior to admission, were excluded from the study. For the Ranson scoring, the scoring criteria of the patients at the first 5 hours and 48 hours were taken into consideration [2]. Scores of 4 and above were considered severe inflammation [2,9]. For APACHE II scoring however, scoring was performed by evaluating the patients' age, certain physiological characteristics and necessary laboratory parameters at 48 hours [3] and a score of 8 and above was considered as a severe case indicator [3,9,10].

All patients underwent cardiac examination, echocardiography (ECHO) and electrocardiography (ECG) prior to treatment within the first 24 hours of admission.

Electrocardiography: All patients had ECG at the time of admission and before treatment. A 12-lead surface ECG during sinus rhythm was obtained from each participant in supine position, following 10 minutes rest with 10 mm/mV amplitude and 25 mm/s paper speed. The QT interval was measured from the first deflection of the QRS complex to the

end of the T wave in as many leads as possible. Hodges formula (QTc= QT+0.00175 ([60/RR]−60)) was used to define corrected QT interval [11]. The Tp-e interval was defined as the interval from the peak of the T wave to the end of it. Measurement of the Tp-e interval was taken from precordial leads. The Tp-e/QT ratio was calculated as Tp-e interval divided by QT interval. Examples for measurement of QT and Tp-e interval presented at Figure 1. The software Cardio Calipers (Iconico Inc., New York, USA) was used for electrocardiographic measurements.

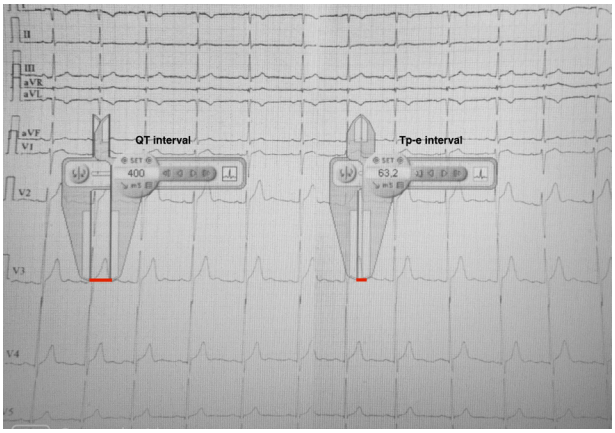

Figure 1. Examples for measurement of QT and Tp-e intervals

Echocardiography: transthoracic echocardiographic examinations were performed in all participants at the left lateral decubitus position to evaluate conventional parameters, using Toshiba, Artida SSH-880 CV equipment (Japan). All echocardiographic measurements were done according to the guidelines of American Society of Echocardiography [12].

Exclusion Criteria were as follows: patients under 18 years of age, pregnant women, those receiving immunosuppressive therapy, regular steroid therapy, patients with choledocholithiasis, hepatic canal stone, obstructive jaundice, cholangitis, those with cholecystitis at the time of admission, those who developed cholecystitis and/or pancreatitis episodes in the last 1 month before admission and patients who underwent ERCP within the last 1 week. In addition, patients with known cardiovascular disease, congenital heart disease, metabolic-endocrine disease, renal disease, hypo-hyperthyroidism, diabetes mellitus, hypertension, acute-chronic respiratory diseases, rheumatologic disorders and use of medications that can affect electrocardiographic parameters, were excluded from the study. Furthermore, ECGs without clearly analyzable QT interval, corrected QT (QTc) interval, peak and the end of the T wave (Tp-e) and Tp-e/QT ratio were also excluded from the study.

The QT interval, QTc interval, Tp-e/QT, Tp-e/QTc ratios of the patients who were included in the study and diagnosed with AP were compared with the control group patients. In addition, these parameters and Ranson, APACHE II and amylase values were taken into account in all patients in the pancreatitis group, and the relationship between the severity of the disease and cardiac parameters was investigated.

Statistical Analysis: Mean, standard deviation, median lowest, median highest, frequency and ratio values were used in descriptive statistics of the data. The distribution of variables was measured using the Kolmogorov-Smirnov test. In the analysis of quantitative independent data, independent samples t test and Mann-Whitney U test were used. The chi-square test was used for the analysis of qualitative independent data, and the Fischer test was performed when the chi-square test conditions were not met. Spearman's correlation analysis was used for correlation analysis and the SPSS 22.0 program was used for the statistical analyses.

Ethical Approval

This study was approved by the Ethics Committee of Alanya Alaaddin Keykubat University of Medical Sciences, all of the subjects' parents were informed regarding the details of the study and signed an informed consent form.

RESULTS

A total of 56 patients with acute pancreatitis were included in the study. Seventeen patients were diagnosed as cholecystitis with pancreatitis, 6 patients were diagnosed with choledochal stone, obstructive jaundice at the time of diagnosis, 1 patient was excluded from the study due to pregnancy and 2 patients were excluded because of previous pancreatitis attacks. The statistical analysis was performed on 60 patients, 30 in the AP patients group and 30 in the healthy control

patients group. The gender distribution of the patients was 28 (46.7%) females, 32 (53.3%) males, and the mean age was 55 (20-90) years. In the disease group, the Ranson score was between 1 and 4, the APACHE II score was between 3 and 10. The Ranson score was 4 (13.3%) in 4 patients, the APACHE II score was 8 and above in 16 patients (53.3%), and the amylase value ranged between 84 and 3010. The age, gender, demographic and medical data of the patients are presented in Table 1.

Age, gender, height, weight and BMI values of the patients in the AP and control groups were not significantly different ($p > 0.05$). There were no statistically significant differences in systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and EF values between the groups ($p > 0.05$). Hb, BUN, creatinine, potassium, Na and Ca values were not significantly different in the AP and control groups ($p>0.05$). There was no significant difference in terms of QTc and QTc values between the AP and control group ($p>0.05$), whereas Tp-e values, Tp-e/QT values and Tp-e/QTc ratios were significantly higher in the AP group compared to the control group ($p<0.05$) (Figure 2). Data for AP and control groups are presented in Table 2.

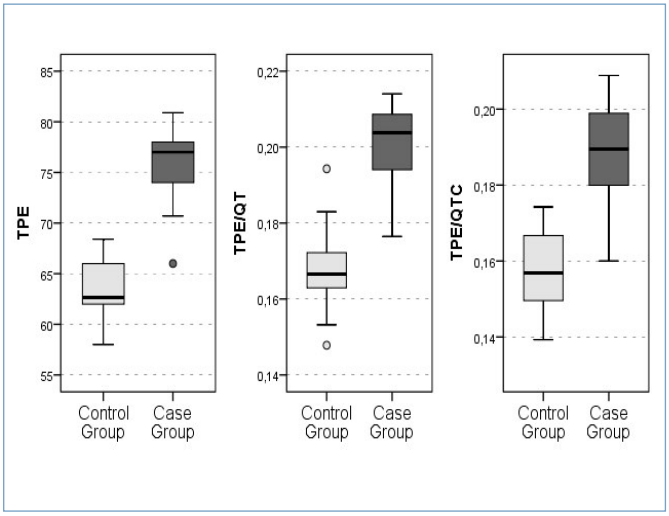

Figure 2. Tp-e interval, Tp-e/QT ratio and Tp-e/QTc ratio were significantly higher in the acute pancreatitis group compared to the control group ($p<0.05$)

Table 1. The age, gender, demographic and medical data of the patients

		Min-Max	Median	Mean±sd/n-%
Age		20,0 - 90,0	55,0	55,1 ± 13,7
Gender	Female			28 /46,7%
	Male			32/ 53,3%
Length		150,0 - 190,0	174,0	173,5 ± 7,2
Weight		52,0 - 120,0	79,0	79,0 ± 14,6
BMI-kg/m^2		17,7 - 38,1	25,3	26,1 ± 4,4
Ranson		0,0 - 4,0	0,5	1,3 ± 1,4
Apache II		3,0 - 10,0	8,0	7,4 ± 1,9
SBP		100,0 - 140,0	120,0	120,8 ± 8,9
DBP		60,0 - 85,0	74,5	73,8 ± 5,0
HR		57,0 - 89,0	72,0	73,4 ± 7,2
Hb		11,9 - 18,0	15,0	14,9 ± 1,2
Bun		5,0 - 20,0	12,2	12,2 ± 3,3
Creatinin		0,6 - 1,1	0,8	0,8 ± 0,1
Potassium		3,6 - 4,3	4,0	4,0 ± 0,2
EF		57,0 - 82,0	68,0	69,7 ± 6,0
Na		133,0 - 146,0	138,0	138,8 ± 2,8
Ca		7,8 - 10,5	9,2	9,2 ± 0,5
QT		350,0 - 402,0	380,0	379,7 ± 13,8
QTc		359,8 - 444,8	403,2	403,1 ± 18,2
TP-e		58,0 - 80,9	68,3	69,7 ± 6,9
TP-e/QT		0,1 - 0,2	0,2	0,2 ± 0,0
TP-e/QTc		0,1 - 0,2	0,2	0,2 ± 0,0
Amylase		84,0 - 3010,0	1653,5	1709,1 ± 1030,2
Cholecystitis	(+)			13/ 43,3%
	(-)			17/ 56,7%

There was no significant correlation between QT and QTc intervals and Ranson score, Apache II score and amylase value ($p>0.05$). There was a significant positive correlation between Tp-e interval and Ranson score, Apache score and Amylase value ($p<0.05$). There was a significant positive correlation between Tp-e/QT ratio and Ranson and Apache II scores ($p < 0.05$), however there was no significant correlation between Tp-e/QT ratio and amylase value. Similarly, there was a statistically significant positive correlation between Tp-e/QTc ratio and Ranson and Apache II scores ($p<0.05$), but no significant correlation was found between Tp-e/QTc ratio and amylase value ($p>0.05$). The correlation analysis between Ranson, Apache II and amylase values and calculated ECG parameters is shown in Table 3.

Table 2. Demographic and medical data of the acute pancreatitis and control group patients

		Control Group	Case Group	P
		Mean±sd/n-% Median	Mean±sd/n-% Median	
Age		55,7 ± 12,9 56,0	54,4 ± 14,6/55,0	0,716 [m]
Gender	Female	14/ 46,7%	14/ 46,7%	1,000x[2]
	Male	16/ 53,3%	16/ 53,3%	
Length		173,9 ± 6,7/174,0	173,0 ± 7,8/ 173,5	0,621 [m]
Weight		81,3 ± 13,0/ 79,5	76,6 ± 16,0/ 77,5	0,217 [m]
BMI-kg/m^2		26,9 ± 4,4/ 25,5	25,2 ± 4,4/ 25,1	0,136 [m]
SBP		118,8 ± 8,5/ 120,0	122,9 ± 8,9/ 120,0	0,101 [m]
DBP		73,8 ± 6,7/ 75,0	73,7 ± 2,4/ 74,0	0,652 [m]
HR		73,8 ± 8,9/ 74,5	73,0 ± 5,1/ 72,0	0,646 [t]
Hb		15,0 ± 0,9/ 15,1	14,8 ± 1,4/ 14,7	0,351 [t]
Bun		12,9 ± 2,5/ 13,0	11,5 ± 3,9/ 11,0	0,095 [t]
Creatinin		0,8 ± 0,1 / 0,8	0,8 ± 0,1/ 0,8	0,891 [t]
Potassium		3,9 ± 0,2/ 4,0	4,0 ± 0,2/ 4,0	0,518 [m]
EF		70,3 ± 5,8/ 69,0	69,0 ± 6,2/ 68,0	0,303 [m]
Na		138,4 ± 2,8/ 138,0	139,1 ± 2,8/139,0	0,238 [m]
Ca		9,3 ± 0,6/ 9,3	9,1 ± 0,5/ 9,1	0,167 [m]
QT		380,6 ±16,8/ 384,5	378,8 ± 10,1/378,7	0,363 [m]
QTc		404,8 ± 22,3/404,4	401,4 ± 13,1/401,8	0,615 [m]
TP-e		63,5 ± 2,9/ 2,7	75,8 ± 3,2/ 77,0	0,000 [m]
TP-e/QT		0,17 ± 0,01/ 0,17	0,20 ± 0,01/ 0,20	0,000 [m]
TP-e/QTc		0,16 ± 0,01/ 0,16	0,19 ± 0,01/ 0,19	0,000 [m]

t :t test / m :Mann-Whitney u test / X^2 :Chi-square test

DISCUSSION

The pathogenesis of the effects of AP on different organs and systems, including the cardiovascular system, can be summarized in 3 stages. The first is the intrapancreatic trypsinogen activation and acinar cell injury as a result of trypsin secretion, the second involves intrapancreatic inflammation reaction development and various degrees of acinar cell necrosis, and the third encompasses development of SIRS and multiorgan dysfunction induced by the destruction of the enzymes, activated following inflammatory cell migration and endothelial adhesion as AP progresses [4]. Clinical studies of the effects of AP on the cardiovascular system have shown changes in the cardiac system such as hemodynamic status (hypovolemia-shock), electrocardiographic changes and pericardial effusion. Studies have shown interstitial edema and hypoxia in cardiomyocytes, increased contractility in myofibers, development of intracellular edema between cardiomyocytes, myocardial stromal collagenization, and cardiomyocyte hypertrophy in patients with AP [13].

Table 3. The correlation analysis between Ranson, Apache II and amylase values and ECG parameters

		Ranson	Apache II	Amylase
QT	r	-0,132	-0,067	0,135
	p	0,313	0,724	0,478
QTc	r	-0,113	-0,142	-0,175
	p	0,388	0,455	0,355
TP-e	r	0,895	0,730	0,618
	p	0,000	0,000	0,000
TP-e/QT	r	0,869	0,507	0,253
	p	0,000	0,004	0,177
TP-e/QTc	r	0,868	0,518	0,450
	p	0,000	0,003	0,012

Spearman Correlation

Electrocardiographic changes may be seen in many patients with AP due to the impact of this disease on the cardiovascular system. In one study, it was reported that 57% of AP patients had transient ECG changes and the most common of these included non-specific ST-T wave changes and atrial or nodal rhythm changes. In this study, it was also found that ECG changes in biliary pancreatitis were more common (80%) than in alcoholic pancreatitis and that this may be due to the fact that biliary pancreatitis is encountered more frequently in older subjects. It has been reported that ECG changes resembling ischemic heart disease findings may be due to stress induced by AP and/or imbalance in the autonomic nervous system [14]. In another study examining 65 patients with AP, the most common ECG change was found to be ST segment depression and T wave inversion with a prevalence of 85%. It was reported that AP could mimic myocardial infarction by causing ECG changes and elevation in several cardiac enzymes [15].

It has been reported that there may be numerous ECG changes including tachyarrhythmia, bradyarrhythmia, supraventricular premature contractions, shortening of PR interval, QRS prolongation, bundle branch blocks such as left bundle branch block, right bundle branch block and left anterior hemiblock, atrial flutter, decrease in T wave voltage, ST segment abnormalities in AP patients and these changes are detected in approximately 50% of the cases [16]. The pathophysiology of ECG changes has been

reported to be associated with myocardial damage caused by pancreatic proteolytic enzymes, vagal autonomic imbalance, metabolic and electrolyte abnormalities, hemodynamic instability, coronary arterial spasm, prothrombotic irregularities and systemic inflammatory response [4].

In our study, QT interval, QTc interval, Tp-e, Tp-e/QT and Tp-e/QTc ratios in patients with AP are the electrocardiographic parameters that can be used as predictors of ventricular arrhythmias. Parameters such as QT interval, corrected QT interval and transmural dispersion of repolarization are used to detect myocardial repolarization by ECG. It is known that QT interval, considered as a cardiac electrical instability marker, is affected by myocardial ischemia, left ventricular dysfunction, neurohormonal activation, electrolyte and metabolic imbalance and some medications. Prolonged QT interval has been reported to increase the risk of arrhythmia and increase the risk of mortality in cardiac and non-cardiac diseases [17]. Tp-e interval which represents the interval between peak and end of T wave in ECG can be used as total (transmural, apicobasal, global) index of repolarization dispersion. In addition, increased Tp-e interval can be used as an indicator of ventricular tachyarrhythmias and cardiovascular mortality. Tp-e/QT ratio, which is a current and new index, has been reported to be a more useful and effective index than QT interval, corrected QT interval and Tp-e interval in determining ventricular repolarization dispersion regardless of heart rate changes [18]. In addition, it was reported that Tp-e interval, Tp-e/QT and Tp-e/QTc ratios were significantly prolonged in patients with slow coronary flow compared to patients with normal coronary flow, and these new indices were positively correlated with reduced coronary flow [19].

Although there are many studies conducted on cardiovascular and ECG changes in patients with AP, according to our review there are no studies in the literature that investigated the new indices, namely Tp-e interval, Tp-e/QT and Tp-e/QTc ratios which can be used as markers of myocardial repolarization and ventricular arrhythmogenesis. In a study performed on QT interval measurement in patients with AP, QTc max and QTc dispersion values were increased in patients with acute biliary pancreatitis compared to healthy individuals, and this was found to be associated with the Ranson score. Furthermore, QT dispersion was increased in parallel with the severity of the disease [17]. In our study, QT interval, QTc interval, Tp-e interval and Tp-e/QT, Tp-e/QTc ratios were examined in patients with acute biliary pancreatitis. While there was no statistically significant difference with regard to the QT and QTc intervals between the AP and control group patients, Tp-e interval, Tp-e/QT and Tp-e/QTc ratios were significantly higher in the AP group compared to the control group. In addition, there was no correlation between the QT and QTc intervals in evaluation performed according to the Ranson and APACHE II scores, which we used to assess the severity of the disease. On the other hand, Tp-e interval, Tp-e/QT and Tp-e/QTc ratios showed a positive correlation with the Ranson and APACHE II scores. In addition to these scores,Tp-e interval displayed a positive correlation with amylase levels. As a result of these findings, Tp-e interval, Tp-e/QT and Tp-e/QTc ratios increased depending on the severity of AP and Tp-e interval increased in parallel with higher levels of amylase.

Although the exact mechanism of the relationship between disease severity and changes in myocardial repolarization and increased risk of ventricular arrhythmia is not known, it has been reported that this relationship may be due to the response of the cardiac system to abnormal proteolitic enzyme levels, vagal reflex, hypovolemia, toxic effects of cytokines and inflammatory mediators, microcirculation insufficiency caused by the increase in complement components and increased vascular permeability [20]. Another cause of QT interval prolongation has been reported to be the increase in the release of catecholamines due to disease and excessive pain and autonomic dysfunction [21,22]. The results obtained on the cardiac effects of AP vary, and it has been reported that left ventricular dysfunction may be due to increased pulmonary vascular resistance in patients with AP [23]. In one study, increased cardiac index was seen in severe necrotizing pancreatitis compared to edematous pancreatitis, but the mean pulmonary vascular resistance was reported to be low in patients with severe AP [24]. In another study, it was reported that prolonged QT interval and diastolic

dysfunction are associated with high mortality in severe pancreatitis and these findings may be used to evaluate the severity of AP [25].

Study Limitations: This study was conducted in a single center. Our sample size was small to conduct a subgroup analysis according to the Ranson criteria. Intra and inter-observer variability analysis would have increased the reliability of ECG measurements.

In conclusion, along with Tp-e interval, one of the parameters that we investigated, new indices, Tp-e/QT and Tp-e/QTc ratios, are sensitive to myocardial repolarization and can be used as a marker for the detection of ventricular arrhythmia risk in various patient groups. Since Tp-e interval is positively correlated with Ranson and APACHE II scores as well as amylase values in patients with pancreatitis, it was concluded that high amylase level alone could be an indicator for increased risk of ventricular arrhythmia in patients with AP. Furthermore, Tp-e interval, Tp-e/QT and Tp-e/QTc ratios can be used as markers to determine the severity of the disease in patients with AP because of the positive correlation of these findings with the severity of the disease.

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Enhanced recovery after surgery (ERAS) and anesthesia

Ameliyat Sonrası Geliştirilmiş İyileşme (ERAS) ve Anestezi

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ABSTRACT

ERAS (Enhanced Recovery After Surgery) is a multimodal approach which aims to optimize perioperative management. ERAS is a combination of changes in preoperative, intraoperative and postoperative care to reduce organ dysfunction and surgical stress response. This concept is managed by multidisciplinary teams that include various areas of expertise to minimize the patient's surgical stress response, optimize physiological functions, and facilitate healing. In order to further increase these developments in various surgical specialties, protocols have been established on this subject and many health institutions offer their services in this way. With the use of ERAS protocols, perioperative and postoperative complications decreased, patient survival and quality of care improved, and patient satisfaction was significantly increased.

Keywords: ERAS, Anesthesia, Surgery.

ÖZ

ERAS (Enhanced recovery after surgery) perioperatif yönetimi optimize etmeyi amaçlayan multimodal bir yaklaşımdır. ERAS, organ disfonksiyonunu ve cerrahi stres yanıtını azaltmak için preoperatif, intraoperatif ve postoperatif bakımdaki değişikliklerin bir bütünüdür. Bu kavram hastanın cerrahi stres yanıtını azaltmak, fizyolojik fonksiyonlarını düzeltmek ve iyileşmeyi kolaylaştırmak amacıyla çeşitli uzmanlık alanlarını içeren multidispliner ekipler tarafından yönetilir. Günümüzde değişik cerrahi uzmanlık alanlarında bu gelişmeleri daha da arttırmak amacıyla bu konu ile ilgili protokoller oluşturulmuş ve birçok sağlık kuruluşu hizmetlerini bu kavrama dayalı bir şekilde sunmaktadırlar. ERAS protokollerinin kullanımı ile periopetif ve postoperatif komplikasyonlarda azalma, hasta sağ kalımında ve bakım kalitesinde artış ve hasta memnuniyetinde belirgin gelişmeler sağlanmıştır

Anahtar kelimeler: ERAS, Anestezi, Cerrahi

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ERAS (ENHANCED RECOVERY AFTER SURGERY) AND ANESTHESIA

ERAS (Enhanced Recovery After Surgery) is a term used to accelerate postoperative recovery, defined by the concept of multimodal perioperative interventions performed by a professional health care team, including current evidence-based medical practices. ERAS started in the 1990s with studies by Prof. Dr. Henrik Kehlet to enhance recovery after colorectal surgery and laid the cornerstones for the formation of protocols, so he is considered the creator of ERAS [1]. Kehlet considered that surgical stress, metabolic and endocrine disorders, and prolonged limitation of motion, caused symptoms such as pain, nausea, vomiting, ileus, loss of strength as well as cognitive dysfunction, and that the severity of organ dysfunction increased and the rate of recovery slowed down. The first results of the study were published two years later [2]. Then, in 2001, a scientific protocol was established by Fearon and Ljungqvist, a working group from Northern European countries (Scotland, Sweden, Denmark, Norway, and the Netherlands) to examine and evaluate the results in accordance with the rules of evidence-based medicine. He worked for a year to develop the protocol, and eventually, a package of recommendations for elective colorectal surgery was prepared and published [3]. In 2010, it was renamed the ERAS Association (www.erassociety.org). Published guidelines for elective colon surgery, rectal surgery, and pancreaticoduodenectomy were followed by other area guidelines (urology, orthopedics, gynecology) in the subsequent years.

ERAS recommends changes related to the entire process that begins in the outpatient clinic of a patient and is discharged after surgery (Figure 1) [4]. The basic philosophy is to reduce metabolic stress resulting from surgical trauma, to support the normalization of functions in a short time, in order to return to normal activity as soon as possible. One of the most important factors in postoperative recovery is the fight against metabolic trauma caused by surgery. ERAS aims to reduce metabolic response to trauma using new surgical techniques, anesthesia, analgesia, and some supportive practices. The less damage the patient has, the faster the recovery will be. ERAS can be done not by a single surgeon but by a trained team of individuals [5, 6]. The surgeon, anesthesiologist and nurses come to the fore, while dietitians and physiotherapists are the other members who complete the team.

ERAS protocols include more than 20 evidence-based elements to be performed during the perioperative period (Table 1) [4,5]. The critical components of ERAS are to inform preoperative patients, make use of short-acting anesthetic agents, limit the use of catheters, drains and tubes, make us of opioid-independent anesthesia, as well as rapid mobilization and feeding. It is not possible to achieve good results by applying only some of the elements contained in the ERAS protocols. When a trained team implements all or at least 80% of the recommendations, their contribution to the postoperative recovery process is improved [5]. The co-application of each element has a better effect than making use of individual parts. The application of ERAS protocols in elective major surgeries has been shown to shorten hospital stay by 2-3 days and reduce complications by 40-50% [7, 8].

Table 1: The basic elements of ERAS protocols [4,5]

Preoperative	İntraoperative	Postoperative
-Patient education -Prehabilitation -Avoid mechanical bowel preparation -Preoperative fasting -Evolution nutritional status and nutritional support -Preoperative optimization -Preoperative medication -Tromboprophylaxis -Antimicrobial therapy -Preparation of surgical side	-Analgesia protocols -Selection of surgical incisions -Avoiding hypothermia -Postoperative nausea and vomiting management -Fluid optimization -Drain management	-No nasogastric tubes -No urinary catheter -Blood sugar management -Stimulation of bowel motility -Postoperative analgesia -Early nutrition -Early mobilization -Discharge planning -Follow up and ongoing support

ANESTHESIA IN ERAS PROTOCOL

According to the ERAS protocol, anesthesia generally includes preoperative preparation and patient counseling, avoiding bowel preparation, giving carbohydrate-rich beverages in the preoperative period, avoiding premedication

with long-acting agents, thromboembolism and antibiotic prophylaxis, use of epidural anesthesia in appropriate cases, use of short-acting anesthetics and opioids in the intraoperative period, restricting parenteral sodium and fluid administration, avoiding hypothermia, preventing postoperative nausea and vomiting as well postoperative pain control with non-opioid techniques, transiting to early enteral feeding, stimulating gastrointestinal motor activity, limiting use of a nasogastric tube, choosing laparoscopic surgery in appropriate cases, ensuring removal of drainage and bladder catheters as early as possible, early mobilization, application of the protocol and evaluation of results [9].

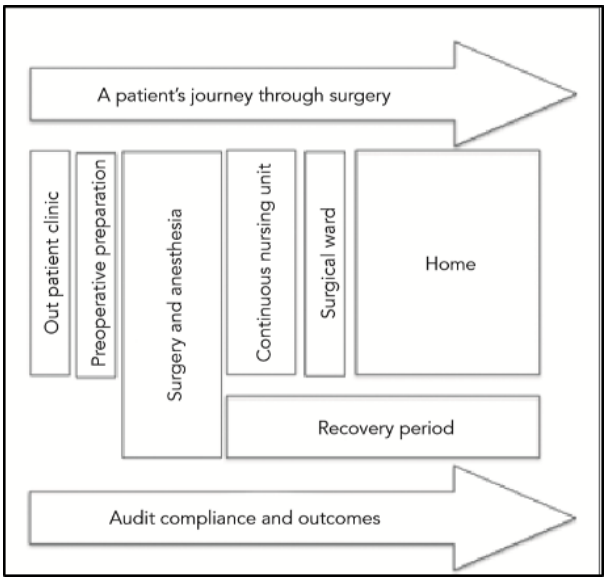

Figure 1: The journey of a surgical patient [4]

Informing the Patient

To implement the ERAS protocol, first of all, the patient should be informed and should comply with the application parameters. Preoperative counseling can alleviate fear and anxiety associated with surgical and anesthesia procedures and accelerate postoperative recovery and discharge. Ideally, the patient should be interviewed with the surgeon, anesthesiologist and nurse before the operation, and informed in writing and orally [10, 11].

Preoperative Optimization

Preoperative care aims to maximize the physical and functional status of the patient and to inform the patient about surgical expectations, cardio-pulmonary preparation, and thus to reduce mortality rates. In order to achieve success in this sense, all patients who undergo major surgery should be operated on after maximizing their general condition. In recent years, preoperative prehabilitation has been developed instead of postoperative rehabilitation [4]. The prehabilitation is a multidisciplinary approach philosophy that will improve the physical condition and nutritional status of patients preoperatively and reduce their anxiety. Accordingly, the patient should stop smoking and using alcohol eight weeks before surgery. Also, exercise programs should be organized, the necessary consultations should be made to reduce the risk of co-morbid diseases and the patient should be operated on after preparing for many similar subjects.

Anemia, smoking, and alcohol use should be adressed before surgery. Studies have shown that interventions for these factors reduce perioperative mortality and morbidity. Smoking is associated with high postoperative risk, however the pulmonary effects of smoking can be improved by quitting four weeks before surgery. Interventions to quit smoking, such as behavioral support and nicotine replacement therapy provide short-term smoking cessation, but the evidence for lower postoperative morbidity is weak [12]. On the other hand, the chronic effects of alcohol on the liver, pancreas and neurological system are known. In the perioperative period, the effects of alcohol on cardiac functions, blood clotting, immune functions, and response to surgical stress have a role in increased morbidity. Intensive preoperative interventions aimed at quitting alcohol at least four weeks in advance to reduce postoperative complications, do not significantly reduce mortality and length of hospital stay [13]. Perioperative anemia is associated with morbidity and mortality and so, should be detected before elective surgery and the underlying disease should be treated.

Although the aim of fasting the patient before elective surgery is to reduce the risk of pulmonary aspiration during intubation, it has been shown that prolonged fasting does not reduce the risk of aspiration. Many studies have shown that prolongation of fasting time leads to an increase in insulin resistance and deterioration in a number of

metabolic conditions. Therefore, the preoperative fasting period should be kept as low as possible. It is recommended that oral intake should be restricted as little as possible, oral feeding with solid foods should be discontinued 6 hours before the operation and clear fluids and water should be administered up to 2 hours [4]. The American Society of Anesthesiologists (ASA) guidelines offer similar instructions. Also, carbohydrate-rich fluids to be applied up to 2 hours before the operation, to ensure metabolic satiety, 800ml until midnight before surgery, and 400ml carbohydrate-rich liquid food should be given before surgery 2-3 hours [14]. Studies have shown that the use of iso-osmolar carbohydrate drinks (400 ml) 2 hours before the induction of anesthesia reduces postoperative hunger, thirst, and anxiety. Besides, the development of insulin resistance will be reduced, and nitrogen, protein loss will be diminished, and lean body mass, muscle mass will be preserved. Preoperative carbohydrate loading has proven to be an independent predictor of clinical outcomes, including postoperative nausea and vomiting [15, 16].

Premedication

A wide variety of agents (such as opioids, benzodiazepines, gabapentin) are currently used to reduce preoperative anxiety. Long-acting premedication agents should be avoided in patients taken with ERAS protocol. Although midazolam has an advantage due to its short effect, it should be kept in mind that its influence can be prolonged by many factors and may prolong the recovery time. Avoiding routine administration is recommended, especially in elderly patients [17]. If necessary, low-acting intravenous drugs may be used in low doses - as they do not significantly affect the compilation - to facilitate epidural or spinal anesthesia.

Anesthesia technique and anesthetic agent selection

In the selection of anesthesia techniques and agents, techniques with minimal side effect profile should be preferred in order to encourage rapid recovery, according to the surgical intervention. The anesthesiologist is responsible for three aspects of influencing the outcome of surgery: stress response to the operation, fluid therapy, analgesia. Understanding the importance of ERAS components has enabled anesthetists to define the “trimodal approach” for the optimization of anesthesia outcomes in laparoscopic surgery [18]. The use of epidural analgesics, in addition to general anesthesia throughout the operation, reduces postoperative intravenous opioid use. Epidural long-acting opioid applications are not recommended because they increase nausea and vomiting. In appropriate cases, thoracic epidural anesthesia-analgesia sympathetic block offers several advantages. In open surgery, higher epidural analgesia is superior to opioid-based alternatives in many situations, including pain, postoperative nausea, vomiting, and complications [19]. Regional block also reduces the stress response and insulin resistance (the primary mechanism of hyperglycemia). Glucose monitoring is essential as hyperglycemia will lead to an increase in postoperative complications [4].

Fluid administration during surgery should be physiological levels and the restrictive fluid application should be preferred; after normovolemia is achieved, it should be corrected with vasopressors to avoid mean blood pressure, fluid, and salt loading. In case of hypotension due to epidural anesthesia, vasopressor agents should be used instead of the fluid application. In these cases, if epidural anesthesia is used, excess fluid should be avoided, especially to maintain the blood flow of the intestine. Local infiltration techniques, regional anesthesia, and peripheral nerve blocks are also preferred in ERAS applications [4].

Agents with a short duration of action and minimal side-effect profiles are generally preferred because they shorten the recovery time in “fast-tracking” applications. Inhalation anesthetics and intravenous hypnotics are commonly used in general anesthesia. The use of short-acting inhalation anesthetics may be advantageous. Propofol is preferred in iv agents due to its short duration of action, its use in induction and maintenance of anesthesia, and its effects on reducing nausea and vomiting after anesthesia. Propofol can be used a dose range of 1.5-2.5 mg/kg in the induction of anesthesia; it can also be used in maintenance anesthesia, a dose range of 4-12 mg/kg/h and target-controlled infusion (TCI) in a dose range of 2-6 µg/mL. Avoiding

profound anesthesia will accelerate recovery [17]. Nitrous oxide is disadvantageous because it increases nausea and vomiting, accumulates in third cavities, and increases thrombotic morbidity [20]. Monitoring of the depth of anesthesia is advantageous as it reduces unnecessary excess drug consumption and recovery. There is new evidence that deep anesthesia can be harmful in elderly patients and increases the risk of postoperative confusion. In these patients, the use of bispectral index monitoring may be useful to keep the depth of anesthesia to a minimum. In the selection of agents, it should be kept in mind that the half-life of opioid infusions may increase with the infusion time. Remifentanil infusion of 0.05-2 μg/kg/min was associated with effective intraoperative analgesia and shortened extubation time following the end of the operation [18]. Neuromuscular blockers are frequently used in general anesthesia practice because of improving intubation and mechanical ventilation conditions as well as improving surgical field quality. The use of short and medium-acting agents in ERAS practice can be chosen to reduce the possibility of postoperative complications and residual block. In addition to delaying recovery, residual block causes an increase in postoperative complications such as desaturation, airway obstruction, and decreased muscle strength. The use of neuromuscular monitoring methods such as “train of four” (TOF) to avoid residual block is important to prevent postoperative complications. Sugammadex, which is used to restore aminosteroid muscle relaxants, is faster and safer than conventional agents, but it should be noted that it increases costs [21].

Antimicrobial prophylaxis should be used as it reduces surgical site infection. The best time for this application is 30-60 minutes before the incision. In prolonged and high blood loss surgeries, it is beneficial to repeat the dose in 3-4 hours, depending on the half-life of the drug. Antibiotic selection depends on local guidelines and should include aerobic and anaerobic bacteria [22, 23].

Postoperative Nausea And Vomiting (PONV)

Postoperative nausea and vomiting affect 25-35% of surgical patients. Nausea and vomiting lead to difficulty in mobilization, delayed discharge, and patient dissatisfaction. The cause of nausea and vomiting may be from the patient, anesthesia, or surgery. Female patients, non-smokers, and those with a history of motion sickness are at risk. The use of inhalation anesthetics, nitrous oxide, and parenteral opioids significantly increases the risk. A high prevalence of PONV was found in 70% of patients who had major abdominal surgery for the colorectal disease [24]. It is stated in the guidelines that the frequency of PONV can be reduced to 40% or less through the use of antiemetics [25]. The scoring system used for PONV is the Apfel score system (Table 2) [26]. These scoring systems reduce the incidence of PONV but have not yet been used in routine clinical practice. Antiemetic prophylaxis is given by a multimodal approach to all patients undergoing major abdominal surgery using inhalation anesthesia and opioids to reduce PONV. Non-pharmacological and pharmacological antiemetic methods are applied together in ERAS programs [27]. Non-pharmacological methods are an avoidance of emetogenic stimuli (such as inhalation anesthetics), use of propofol in induction and maintenance of anesthesia. Keeping the preoperative fasting period short, carbohydrate loading and sufficient hydration is also useful. The use of regional anesthesia techniques reduces PONV by reducing the need for postoperative opioids. NSAID and paracetamol use are also recommended as an alternative to opioid use. The effects are increased with the combined use of two or more antiemetics.

Table 2. Apfel Nausea Vomiting Scoring [26]

Risk factors	Points
Female gender	1
Non- smoker	1
History of PONV	1
Postoperative opioids	1

PONV: Postoperative nausea and vomiting

Early Mobilization, Early Nutrition

Early mobilization may counteract insulin resistance due to immobilization by reducing lung complications [28]. Although studies are supporting the direct beneficial effects of postoperative mobilization, prolonged immobilization is associated with an increased risk of pneumonia, insulin resistance, and muscle weakness. Providing early postoperative mobilization is

also important to avoid pain and ileus. Patients are advised to spend outside the bed for 2 hours on the day of surgery and 6 hours per day until discharge.

Postoperative early feeding is one of the essential components for postoperative well-being and early discharge. The perioperative nutrition management algorithm recommended for patients to be operated according to ERAS protocols begins with a routine nutritional assessment and proceeds at each stage by targeting oral/enteral nutrition (Figure 2) [29]. With three components of ERAS - preoperative carbohydrate intake, epidural analgesia, and early enteral nutrition - nitrogen balance is maintained without minimal insulin resistance and hyperglycemia [30]. In the ERAS protocol, oral fluid intake should be encouraged immediately after the patient awakens from anesthesia. With early enteral feeding, there is a reduced risk of hospitalization and infection without increased anastomotic leakage.

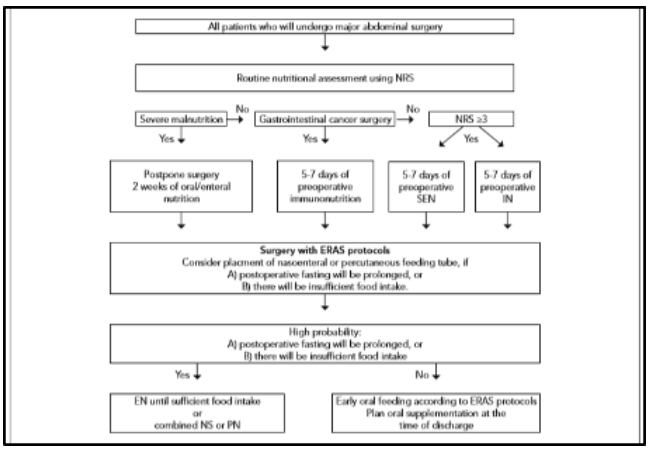

Figure 2: Perioperative nutritional planning algorithm [29]. (NRS: Nutritional Risk Score NS: Nutritional IN: Immuno Nutritional SEN: Surgical Enteral Nutrition PN: Parenteral Nutrition EN: Enteral Nutrition)

Meta-analyses also showed that clinical benefits of immuno-nutrition, including arginine, glutamine, omega-3 fatty acids, and nucleotides. In patients with malnutrition, supportive (oral and parenteral) treatment initiated 7-10 days before surgery has been observed to reduce complications [31]. Enteral solution administration should be continued for at least eight weeks postoperatively.

Postoperative Analgesia

Ideal analgesia regimens after major surgery should relieve pain, facilitate early mobilization and return bowel movements, to help switch to oral nutrition, and should not cause complications. In upper abdominal open surgery, TEA (Tx 5-8 level-thoracoepidural analgesia), in which short-acting opioid and local anesthetic agents are combined in the first 48-72 hours postoperatively, should be preferred. According to meta-analyses, compared with opioid-based analgesia, TEA produced better results in pain control, complications, prevent postop nausea and vomiting, and decreased insulin resistance. The most commonly used alternative method to TEA is the TAP block (transversus abdominis plane block). This method is a local anesthetic injection between the internal oblique and transversus abdominis muscles under ultrasound guidance. TAP block is associated with less narcotic use, low pain score, and decreased postoperative nausea and vomiting. The multimodal analgesia regimen includes Paracetamol and NSAIDs. Paracetamol 4 mg / day is sufficient [32].

Discharge

According to the ERAS protocol, the following criteria must be met if the patient is to be discharged: the need for intravenous fluid should be eliminated and the patient should be able to take enough food orally, pain control should be achieved with oral analgesics, adequate mobilization should be possible, intestinal functions should return, infection signs and symptoms should not be present, and comorbid diseases should be controlled. Patients sent home should be contacted by telephone after 24 - 48 hours, and their status should be noted. If there are no problems, the patient should be invited to control the wound and remove the sutures on postoperative days 7-10. Since the pathology report will also be prepared during this period, additional oncologic treatment should be planned if necessary. Anastomosis leakage or other major complication should be kept in mind in 1-3% of the patients taken home, and every complaint should be carefully examined. The next interview can be done by telephone on the 30th postoperative day [4].

To increase the success rates of these goals in different clinical applications and branches, it is vital to reveal the factors and mechanisms that

most affect recovery. To increase patient safety and medical practice success in ERAS applications specific to various surgical branches and different surgical types, and to decrease complications, costs, and length of hospital stay are being made in many new clinical studies. Successful implementation of ERAS protocols require the cooperation of the surgery, anesthesia, nursing and nutrition departments.

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REVIEW

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Quartile scores of scientific journals: Meaning, importance and usage

Bilimsel Dergilerin Q Değerleri: Anlamı, Önemi ve Kullanımı

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ABSTRACT

The Q scores of scientific journals are an analytical tool that determines the ranking of journals based on their own scientific group and impact factor. It shows the rankings of the journal within its own group and it is a useful guide for researchers in the selection of the journal, however it should be used with caution in academic career advancement. Quartile scores may vary according to the scientific categories. The Q scores of a journal briefly show the 25% quantile resulting from quartered the number of journals in the area where the journal is placed. The first quartile has the top 25% of the journals and gets the Q1 score and the last quartile gets Q4 score. And so, the second 25% slice takes Q2 score and the third 25% slice takes Q3 score.

ÖZ

Bilimsel dergilerin Q değerleri, dergilerin kendi grubunda ve etki faktörüne bağlı sıralamasını belirleyen analitik bir araçtır. Derginin kendi grubu içindeki sıralamasını gösterir, yararlıdır ve akademisyenlerin dergi seçiminde de yol göstericidir ancak akademik yükseltmelerde kullanılmasında dikkatli olunmalıdır. Q değerleri bilimsel alanlara göre değişiklik gösterebilir. Bir derginin Q değeri kısaca, derginin bulunduğu alandaki dergi sayısının dörde bölünmesiyle ortaya çıkan% 25'lik dilimleri gösterir. İlk% 25'lik dilim Q1 değerini alırken, son% 25'lik dilim Q4 değerini alır. Dolayısıyle ikinci% 25'lik dilim Q2, üçüncü% 25'lik dilim ise Q3 değerini alır.

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INTRODUCTION

Various analytical sources are used to measure the output of scientific journals, authors and institutions, etc. One of the most well-known of these is the impact factor (IF) of journals. The impact factor term is related to the citations of full text and review articles published in a journal, per full text or review articles, in the following years. The calculation of the impact factor is based on the performance of the scientific journal in the last three years. Calculation of impact factor can be explained with an example: For instance, a journal publishes 80 articles (x) in two years (2017 and 2018) and these 80 articles receive 160 citations (y) in 2019. Therefore, the impact factor score of this journal for 2019 will be (y/x)= 160/80= 2. Namely, the last three years are always taken into consideration for the calculation of impact factor, but 5-year impact factors can also be calculated and published every year in the Web of Science database. The high number of citations received per article increases the impact factor scores. However, articles that do not receive potentially citations may increase the number in the denominator and may decrease the impact factor score [1-3].

Why did we begin with the impact factor score when trying to explain the Q scores of scientific journals? Because the Q scores are more directly related to the impact factor scores.

Calculation and Quartile Scores of Scientific Journals

The Q score is derived from the English word “quartile” and signifies a quarter. To calculate the Q score of a journal, it is necessary to know the number of journals in the category placed in the said journal. As in the Web of Science [4] database, journals covered by SCI-Expanded are grouped by scientific category; without this grouping, the Q score of journals cannot be calculated. For example, there are 40 journals in the category x and 96 journals in the category y. The impact factor scores of the these journals for the last year are determined. We can explain the Q score with an example: If there are 120 journals in category z covered by SCI-Expanded database: 120 journals ranked from 1 to 120 according to the impact factor scores. The number 120 is divided by four, and four quarters are obtained for taking Q scores (Table 1).

Journal Score	Definition
Q1	Top 25% of the highest impact factor score of journals in a scientific category.
Q2	Second, 25% of the highest impact factor score of journals in a scientific category.
Q3	Third, 25% of the highest impact factor score of journals in a scientific category.
Q4	The last 25% of the highest impact factor score of journals in a scientific category.

Thirty journals in the first category with the highest impact factor score are Q1, the second 30 journals are Q2, the third 30 journals are Q3, and the last 30 journals are Q4. More information on this can be found on the website [5] and even further information can be found on this subject in Tonta's article [6]. Q scores of journals are given in 25% slices, but the number of publications in journals is not compatible with this. For example, Liu et al. stated that 33.33% (1/3) of the publications were published in Q1 journals and 16.5% in Q4 journals, according to the JCR 2015 [7]. However, sometimes a journal can have more than one Q score because of a journal can sometimes participate in more than one scientific category [7].

Meaning and Usage of Quartile Scores of Scientific Journals

The Q scores of the journals give us information about the citation performance of given journal and its place in the community of journals in the given scientific category. In addition, researchers may use the Q scores to inform themselves of the status of the journal when selecting a journal for submission of a manuscript. However, when an academician is appointed to the academic personnel, the situation changes if some institutions ask which Q score journals this academician publishes his papers in. If a journal has more than one Q score, which Q score should be considered by the employer? Taking into account the highest Q score can be rational and fair, however if the person publishes a paper(s) in the x scientific category and the Q score of the y scientific category is taken into consideration, it will result in a score being attributed to this person's publication, though the article in question

is not, in fact, in the y scientific category. Therefore great care should be taken where an academician may receive points for academic progress from a scientific category where they are actually not published in.

Q scores of journals are generally available from databases such as SCImago and Web of Science [4,8]. Q scores of the journals for the last year can be accessed via the latter and SCImago also provides Q values for the previous years, however the Q scores of a journal may be different depending on the database consulted because the number of journals covered is different, and as a result the number of citations of journals will also be different. Here are examples to illustrate this fact: according to information found on the website SCOPUS, the Academic Radiology Journal was Q1 for the year 2016, while for the same year in the Web of Science database it was Q2. In the same way, the Journal of Cancer Education covered in SCOPUS for 2018 was in the category of oncology 215/320 (Q3), while it was ranked 203/230 in the category of oncology for 2018 (Q4) in the Web of Science. These indicate that the Q scores of journals may differ from one database to another [9].

If we only take the Web of Science database into account, the Q scores of journals can be found for the last year only (for example, if we are in the 2019 year, the latest available data is from 2018). In this case, which Q score will the academician consider to be nominated? Inversely, if taking the Web of Science into consideration is mandatory and an academician cannot access the Q value of the journal for a paper, for example, published in 2015, he/she must take into account the last Q score available, say 2018. Invariably, Q scores of journals may differ from year to year and a journal with Q4 score in 2015 may be attributed Q2 or Q3 in 2018, and in such a case the scoring may be incorrect. Furthermore, Q scores also can vary in themselves. For example, if there are 200 journals in any scientific category (for example, there are 203 journals in the Web of Science of surgery category), so 200/4 = 50, would be 50 journals per 25% slice, meaning there are 50 journals in Q1. This number also means that both the first and the 50th journals are in the Q1 category. However, impact factor scores of the first journal and 50th journal are different and thus a researcher who publishes in the journal ranked 50th and a researcher who publishes in the journal ranked 1st will receive the same score. Another issue is that the journal ranked 51st will receive a Q2 score, however the difference between 50th and 51st journals is usually very narrow. This can be explained through an example: according to the Web of Science 2018 data, there are 223 journals in the category of oncology. Among these journals, the journal with the impact factor score of 223.679 in 2018 and in the Q1 category is Ca-A Cancer Research for Clinicians. The impact factor score of Cancer Science, which is 57th in the oncology category and is also Q1, is 4.751. In this case, the paper of an academician published in Ca-A Cancer Research for Clinicians and the paper of an academician published in Cancer Science will receive the same score, because they are both in the Q1 category. However, the impact factor score of the first journal is an extraordinary score of 223.679, whereas the impact factor score of the 57th journal is 4.751, which are considerably different. There is in fact a 47.08-fold difference in the impact factor score between the two journals. Here, not only the 57th rank but as an example, the Annals of Oncology, ranked 9th - with a 14.196 impact factor score - is far behind the impact factor score of the first-ranked journal. The American Journal of Cancer Research ranked 58th in the oncology category, is in the Q2 category because of its impact factor score of 4.737 for the year 2018. However, the difference between the impact factor score of the 57th journal and the impact factor score of the 58th journal is only 0.014 though persons who published papers in these two journals will receive different scores or points. As the number of journals in a scientific category increases, so also increases the difference in the impact factor score. As an example of this effect, the Web of Science biochemistry-molecular biology scientific category has 299 journals for the year 2018: accordingly, in terms of impact factor score, the journal in the 1st and 75th rank will receive Q1 score and be calculated with the same score. However, impact factor scores of the first and 75th journals are not equal [10].

As the number of journals in one scientific category decreases, the difference decreases as well. In short, as the scientific category is

specific, the number of journals in the scientific category decreases and the number of journals in the scientific category increases, as the scientific category becomes general. For example, according to 2018 data in the Web of Science database, there are 27 journals in allergy, 29 in mycology, 76 in orthopedics, 128 in immunology, and 160 in general medicine. That is to say, the number of journals are increasing progressively towards the general scientific category [10]. Campanario indicated the way self-citations of 51 selected journals from the JCR-SCI field affect the journal's impact factor and Q scores [11]. Tayyab and Boyce reported that the impact factor scores of 39 journals selected from different scientific disciplines in the Q1 category ranged from 0.611-1.979 (below 2), whereas the journals in the biochemistry-molecular biology category were at least 4.405 and above [12]. In other words, impact factor and Q scores of journals vary according to the scientific category. According to the information provided by Tayyab and Boyce's article, the journal in each Q1 category may not have a high impact factor score [12]. Miranda and Garcia-Carpintero have already indicated that the Q scores of journals differ according to the scientific categories [13].

Status of Turkey's Scientific Journals:

Q scores of journals are used as academic incentives in Turkey [14]. The Q categories of Turkey's Journals show that we need to make more efforts in this regard as unfortunately, there are no journals in the Q1 category among our journals covered by SCI-Expanded and SSCI [15]. We presented Q scores of Turkish journals covered by SCI-Expanded, SSCI and SCIMago-SCOPUS in Table 2. There is no any journal in category Q1 covered by SCI-Expanded and SSCI databases but there are 5 journals in category Q1 in SCIMago-SCOPUS because of number of journals is different than in the previously mentioned databases (Table 2). Two journals (Atmospheric Pollution Research, Turkish Journal of Agriculture and Forestry) are in category Q2 in SCI-Expanded and SSCI databases, others are category Q3 and Q4. Data presented in Table 2 are the latest, though new data (2019) is expected to be published in June or July, 2020.

The Q scores of scientific journals provide valuable information in terms of citation and ranking in their scientific categories, and they are beneficial. They can also be useful for the selection of journals for academicians to submit manuscripts to, and can guide them adequately in this regard. However, the use of the Q scores of the journals for the purpose of academic staff appointments have the potential of being misinterpreted, and therefore caution is warranted and recommended.

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Table 2. Q Scores of Journals Originated from Turkey.

Title of Journal (alphabetical order) (Total: 13,608 journals; 63 journals are originated from Turkey)	Q Score of Journal (Web of Science Database) (2018)	Q Score of Journal ** (SCIMago by SCOPUS Database) (2018)
Covered by SCI-Expanded		
Acta Orthopaedica Et Traumatologica Turcica	Q4 (64 of 76)	Q3
Anadolu Psikiyatri Dergisi – Anatolian Journal of Psychiatry	Q4 (144 of 146)	Q4
Anatolian Journal of Cardiology (Former title: Anadolu Kardiyoloji Dergisi/The Anatolian Journal of Cardiology	Q4 (122 of 136)	Q3
Ankara Universitesi Veteriner Fakultesi Dergisi	Q4 (122 of 141)	Q3 (Veterinary, Misc.). Q4 (Animal Sci-Zoology)
Archives of Rheumatology (Former title: 2: Turkish Journal of Rheumatology (1: Romatizma–Rheumatism)	Q4 (30 of 31)	Q4
Atmospheric Pollution Research	Q2 (90 of 251)	Q1 (Waste Management and Disposal). Q2 (Atmosphric Sci, Pollution)
Balkan Medical Journal (Former title: Trakya Universitesi Tip Fakultesi Dergisi)	Q3 (94 of 160)	Q3
Diagnostic and Interventional Radiology	Q4 (97 of 129)	Q2
Eklem Hastaliklari ve Cerrahisi / Joint Diseases and Related Surgery	Q4 (Orthopedics: 58 of 76). Q4 (Surgery:163 of 203)	Q2 (Rehabilitation). Q3 (Surgery; Orthopedics; and Sports Medicine).
Experimental and Clinical Transplantation	Q4 (25 of 25)	Q3
Hacettepe Journal of Mathematics and Statistics	Q3 (Mathematics: 207 of 314).Q4 (Statistics & Probability:105 of 123)	Q3
Isi Bilimi ve Teknigi Dergisi – Journal of Thermal Science and Technology	Q4 (Engineering, Mechanical: 126 of 129). Q4 (Thermodynamic: 58 of 60)	Q2 (Engineering). Q3 (Materials Science). Q4 (Atomic and Molecular Physics, and Optics)
Journal of Agricultural Sciences-Tarım Bilimleri Dergisi	Q4 (54 of 57)	Q4
Journal of Clinical Research In Pediatric Endocrinology	Q3 (Pediatrics: 85 of 125). Q4 (Endocrinology & Metabolism:131 of 145)	Q2 (Pediatrics, Perinatology and Child Health). Q3 (Endocrinology; Diabetes and Metabolism)
Journal of International Advanced Otology (former title: Mediterranean Journal of Otology)	Q4 (40 of 42)	Q2 (Medicine). Q3 (Otorhinolaryngology)
Journal of Neurological Sciences-Turkish	Q4 (267 of 267)	Q4
Journal of Sports Science and Medicine	Q3 (50 of 83)	Q1 (Orthopedics and Sports Medicine; Physical Therapy, Sports Therapy and Rehab). Q2 (Sports Science)
Journal of the Entomological Research Society	Q4 (98 of 98)	Q4
Journal of the Faculty of Engineering and Architecture of Gazi University	Q4 (76 of 88)	Q1 (Architecture). Q2 (Engineering, Misc.)
Kafkas Universitesi Veteriner Fakultesi Dergisi	Q4 (111 of 141)	Q3
Mikrobiyoloji Bulteni	Q4 (130 of 133)	Q3 (Microbiology, Medicine, Inf. Disease). Q4 (Immunology and Microbiology)
Neurological Sciences and Neurophysiology	* (Because of covered by SCI-Expanded since Dec.25, 2018)	*
Noropsikiyatri Arsivi-Archives of Neuropsychiatry	Q4 (189 of 199)	Q3 (Psychiatry and Mental Health).Q4 (Neuroscience)
Psychiatry and Clinical Psychopharmacology (Klinik Psiko-farmakoloji Bulteni–Bulletin of Clinical Psychopharmacology)	Q4 (Pharmacology & Pharmacy: 259 of 267). Q4 (Psychiatry 141 of 146).	*
Records of Natural Products	Q3 (Chemistry,Applied:48 of 71). Q3 (Chemistry, Medicinal:53 of 61): Q4 (Plant Sciences:138 of 228):	Q2 (Plant Science). Q3 (Pharmacology; Organic Chemistry; Drug Discovery)

Table 2. Continued

Teknik Dergi	Q4 (131 of 132)	Q4
Tekstil ve Konfeksiyon	Q4 (23 of 24)	Q3
Turk Gogus Kalp Damar Cerrahisi Dergisi-Turkish Journal of Thoracic and Cardiovascular Surgery	Q4 (202 of 203)	*
Turkish Journal of Agriculture and Forestry	Q2 (32 of 89)	Q1 (Forestry). Q2 (Agriculture; Ecology)
Turkish Journal of Biochemistry–Turk Biyokimya Dergisi	Q4 (296 of 299)	Q4
Turkish Journal of Biology	Q4 (76 of 87)	Q3 (Agriculture&Biological Sci) Q4 (Cell Biology; Genetics; Microbiology; Mol.Biology)
Turkish Journal of Botany	Q3 (152 of 228)	Q2
Turkish Journal of Chemistry	Q3 (Engineering, Chemical:103 of 138). Q4 (Chemistry,137 of 172)	Q3
Turkish Journal of Earth Sciences	Q3 (145 of 196)	Q2
Turkish Journal of Electrical Engineering and Computer Sciences	Q4 (Computer Science, Artificial Intelligence:125 of 134). Q4 (Computer Science, Artificial Intelligence:125 of 134)	Q3
Turkish Journal of Field Crops	Q4 (69 of 89)	Q2
Turkish Journal of Fisheries and Aquatic Sciences	Q4 Fisheries:43 of 52).	Q3
	Q4 (Marine Biology: 92 of 108)	
Turkish Journal of Gastroenterology	Q4 (81 of 84)	Q3
Turkish Journal of Geriatrics-Turk Geriatri Dergisi (+SSCI)	Q4 (Geriatrics & Gerontology:53 of 53).Q4 (Gerontology:35 of36).	Q4
Turkish Journal of Hematology	Q4 (70 of 73)	*
Turkish Journal of Mathematics	Q3 (211 of 314)	Q3
Turkish Journal of Medical Sciences	Q4 (138 of 160)	Q3
Turkish Journal of Pediatrics	Q4 (123 of 125)	Q3
Turkish Journal of Physical Medicine and Rehabilitation	Q4 (65 of 65)	Q4
Turkish Journal of Veterinary & Animal Sciences	Q3 (104 of 141)	Q3
Turkish Journal of Zoology	Q4 (143 of 170)	Q3
Turkish Neurosurgery	Q4 (Clinical Neurology:187 of 199). Q4 (Surgery:175 of 203)	Q3
Turkiye Entomoloji Dergisi-Turkish Journal of Entomology	Q3 (70 of 98)	Q3
UHOD-Uluslararasi Hematoloji-Onkoloji Dergisi	Q4 (204 of 230)	Q4
Ulusal Travma ve Acil Cerrahi Dergisi- Turkish Journal of Trauma & Emergency Surgery	Q4 (24 of 29).	Q3
Covered by SSCI		
Amme Idaresi Dergisi	Q4 (46 of 47)	Q4
Bilig	Q4 (72 of 74)	Q3
Egitim ve Bilim–Education and Science	Q4 (216 of 243)	Q3
New Perspectives on Turkey	Q3 (65 of 104)	Q1 (Cultural Studies; History). Q3 (Sociology and Political Sci; Economics- Econometri)
Turk Psikiyatri Dergisi	Q4 (136 of 142)	*
Turk Psikoloji Dergisi	Q4 (136 of 137)	Q4
Turkish Journal of Geriatrics-Turk Geriatri Dergisi	Q4 (Geriatrics 53 of 53). Q4 (Gerontology: 35 of 36).	Q4
Uluslararasi Iliskiler–International Relations	Q4 (91 of 91)	Q4

Table 2. Continued

Covered by AH&CI (Clarivate Analytics do not publish impact factors of AH&CI journals)		
Adalya	*	**Q2 (Archeology, Conservation, History). Q3: (Archeology) (2017)**
Belleten	*	**Q3 (History). Q4 (Cultural Studies)**
METU Journal of The Faculty of Architecture	*	**Q2 (Architecture)**
Milli Folklor	*	**Q2 (Cultural Studies). Q3 (Art and Humanities)**
Olba	*	**Q4 (Archeology)**
Osmanli Arastirmalari-The Journal of Ottoman Studies	*	**Q3 (Cultural Studies, History)**

*** No data**

****https://www.scimagojr.com/ (Total: 31,971 journals; 219 journals are originated from Turkey)**

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LETTER TO EDITOR

EDİTÖRE MEKTUP

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Predatory Congress

Yırtıcı Kongreler

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To the Editor,

I read with great interest the article by Dr. Aslan with reference to his experience with predatory journals and publishers in view of the literature, published in Acta Med. Alanya 2018;2(3):136-137 [1]. The author described an serious issue that leads to a confusing situation when investigators are reviewing publication entities and I thank the author for this important contribution in clarifying a crucial topic that was lacking definitive data and that had received insufficient attention. Moreover, the other way of presenting scientific data, through scientific meetings, is also the source of some controversy. Indeed, as the final step for scientific studies and research results to be shared with fellow scientists and the scientific community at large, they are submitted to the academic congresses, in the related branch of science, leading to the publication of the final manuscript. In recent years, journals, congresses and similar academic entities have been found to be predatorial in their interests in commercial gain and academic promotion [1,2].

As a result of changes in laws and regulations in our country, it has become obligatory for Doctor Lecturer, Associate Professor, and Professorship assignments to make presentations to a certain number of national and international Congresses. In particular, points and financial gains can be obtained from these congress presentations, within the scope of the academic promotion system of the Higher Education Council and the Ministry of Health. To illustrate this point, it has come to light in recent years that the organizations will present the articles and allow oral presentations, in exchange for the payment of a fee [3]. The aim of the existence of these organizations is to meet the demands of multidisciplinary science, not to favor one particular scientific field in exchange for a fee [4]. They are supposed to perform activities that include international book printing and ISBN numbering [5]. However, the predatory practices of these journals, symposiums and congresses do not contribute to the progress and development of science; their function of gathering the relevant science branches, discussing ideas, presenting

and discussing new scientific data, is not being performed [6].

In recent years, these practices, which have threatened the scientific community, have been recognized by the Higher Education Council; restrictions have been placed on predator journals and additional measures are on the agenda for the near future. This hazard has been reported in the literature [7,8] and although such congresses and journals may appear to contribute to the authors in the short term, they pose a great danger to them in the long-term: these money-oriented organizations, which seek only to benefit themselves and their associated magazines have no ethical concerns, which can create catastrophic problems for the authors.

The damage of predatory practices to the scientific community and their repercussions in international literature by congress organizations, are of grave concern; the Acta Medica Alanya: Journal of Alanya Alaaddin Keykubat University Faculty of Medicine would like to draw attention to this rising threat and its associated consequences.

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