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Comparison of Belt and Suspender's Technique with simple double-row rotator cuff repair in patients with chronic rotator cuff tear

Kronik rotator manşet yırtığı olan hastaların tedavisinde basit çift-sıra rotator manşet tamiri ile "Belt and Suspender's" tekniğinin karşılaştırılması

İsmail Türkmen [1]

Abstract

Aim: Surgical treatment of chronic rotator cuff tears is still unclear. Many surgical techniques have been used in the treatment. The aim of the study is to compare the functional outcomes of two different surgical techniques.
Methods: Of the 27 patients in the study; 13 underwent Belt and Suspender's Technique (2 male, 11 female), 14 underwent simple double row rotator cuff repair technique (4 male, 10 female). Clinical assessment was made according to American Shoulder and Elbow Surgeons (ASES) scoring system and Visual Analog Scale (VAS).
Results: There was no statistically significant difference between postoperative ASES scores, VAS scores, range of motions (ROM) and complications. In both groups, failure of healing was seen in one each patient.
Conclusion: According to this study treatment for chronic rotator cuff tear using a Belt and Suspender's technique is as effective and reliable as simple double row cuff repair technique method with low complication rates and good results can be achieved in clinical outcomes in the early postoperative period.
Key Words: Chronic rotator cuff tear, double-row repair, functional outcome, Belt and Suspender's technique.

Öz

Amaç: Kronik rotator manşet yırtıklarının tedavisi henüz açıklığa kavuşmamıştır. Birçok cerrahi teknik kullanılagelmiştir. Bu çalışmanın amacı farklı iki cerrahi tekniğin fonksiyonel sonuçlarının karşılaştırılmasıdır.
Yöntem: 27 hastanın 13'üne (2 erkek, 11 kadın) Belt and Suspenders tekniği, 14 'üne (4 erkek, 10 kadın) klasik çift-sıra tekniği ile rotator manşet tamiri uygulanmıştır. Klinik değerlendirme American Shoulder and Elbow Surgeons (ASES) skorlama sistemi ve ve Görsel Analog Skala (VAS) kullanılarak yapılmıştır.
Bulgular: Ameliyat sonrası dönem ASES ve VAS skorları arasında, eklem hareket açıklıkları arasında ve komplikasyonlar bakımından anlamlı farklılık görülmedi. Her iki grupta da birer hastada iyileşme de yetersizlik görüldü.
Sonuç: Çalışmamıza göre Belt and Suspender's tekniği en az basit çift sıra rotator manşet tamiri kadar etkili ve güvenilirdir. Ameliyat sonrası erken dönemde iyi fonksiyonel sonuçlar elde edilebilir.
Anahtar Kelimeler: Kronik rotator manşet yırtığı, çift-sıra tamir, fonksiyonel sonuç, "Belt and Suspender's" tekniği.

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Introduction

Rotator cuff creates most of the shoulder joint [1]. The shoulder may be exposed to trauma because it is one of the most active joints of the body. In addition, rotator cuff tears can occur spontaneously after middle age and become an important cause of shoulder pain. Chronic rotator cuff tears can be very painful and are a common cause of limited shoulder function [2, 3].

Conservative and surgical treatment methods are applied in rotator cuff tears. Surgical approaches in rotator cuff tears in the world and in our country are rapidly developing and diversifying. There are a few options for repairing of chronic rotator cuff tears [4, 5].

The aim of this study is to evaluate the results and effectiveness of two different techniques of arthroscopic repair on patients with chronic rotator cuff tear and to contribute to the literature on its advantages, challenges and complications.

Material and methods

This study was approved by the ethics committee of the Umraniye Training and Research Hospital. Informed consent was obtained from all patients who participated in the study. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patients with Patte class 2 rotator cuff tear in the shoulder MRI who referred to the clinic with a shoulder pain complaint and underwent arthroscopic rotator cuff repair with two different double-row techniques identified as the target group. Patients were evaluated retrospectively by being invited to the outpatient clinic.

Inclusion criteria were; patients having double row repair for chronic rotator cuff tear, being 40-70 years of age, having at least six months time period after surgery.

Exclusion criteria were; having open surgery, patients with massive-irreparable rotator cuff tear, patients out of standard rehabilitation criteria, individuals having fatty atrophy in their rotator cuff muscles.

Surgical indications were presence of a chronic rotator cuff tear which non-operative treatment methods; pain medication and physical therapy were failed.

Clinical Evaluation

Patients were evaluated before and after the operation in terms of physical examination with the American Shoulder and Elbow Surgeons (ASES) scoring system (highest 100 points) and clinical and functional outcomes were assessed [6]. In the last control, the range of motion was measured with a goniometer in the patient sitting position. Muscle strength was assessed manually. Patients with unexplained shoulder pain at last follow-up were evaluated with shouldermagnetic resonance imaging (MRI).

Surgical technique

All surgeries performed by one surgeon (I.T.). All patients were operated under general anesthesia, in the beach chair position, with standard anterior, posterior, anterolateral and posterolateral portals opening after 1-gram cefazolin sodium prophylaxis. No patient underwent block anesthesia. In addition to the routine glenohumeral joint examination, the rotator cuff was examined. All the patients underwent biceps tenotomy and subacromial decompression. Rotator cuff tears were treated with two different techniques.

Belt and Suspender's technique

First, a medial row was created by placing a metal anchor (5.0 mm titanium anchor, Twinfix, Smith and Nephew, USA) (including two white and two blue threads). One blue and one blue white were passed upwards through the cuff tear by suture passer and these were brought out the lateral cannula tied together, and then the un-tied white and blue sutures were tensioned which brings the knot over cuff tear (this is the belt or the transverse limb); remaining untied sutures were brought up through the cuff on either side of the transverse limb, and were then passed through a knotless anchor which forms the lateral sided fixation; (these form the suspenders) when the knotless anchor was tensioned, not only were the suspenders tensioned but also the belt was tensioned (Figure 1, 2) [7-11].

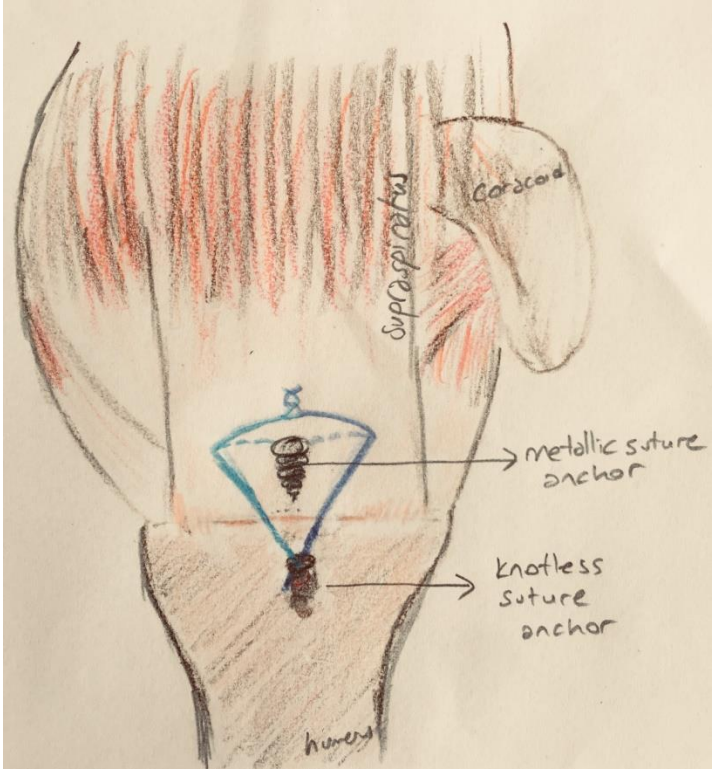

Figure 1. Schematic drawing representing Belt and Suspender's technique (axial view).

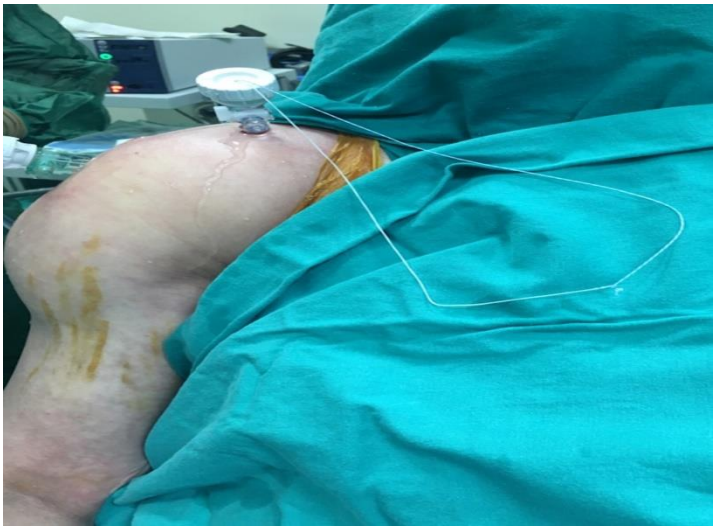

Figure 2. One blue and one white strand tied out of anterior portal (Belt and Suspender's technique) (anterior view).

Simple double-row technique

First, a medial row was created by placing a metal anchor (including two white and two blue threads). Two blue and two white were passed through the cuff and these four were brought out the lateral canula together without ant tying and than passed through a knotless anchor thus the lateral row was created (Figure 3).

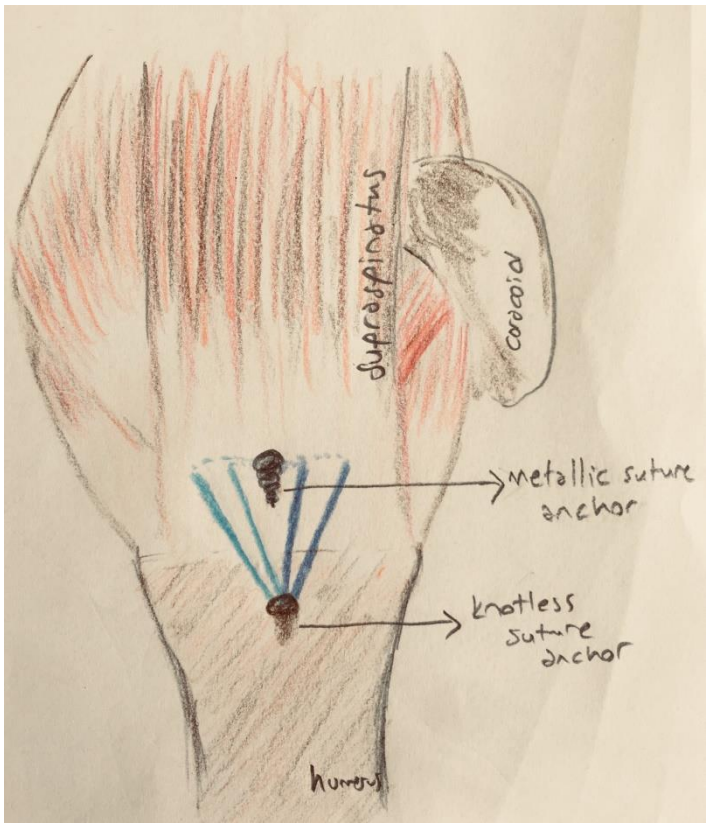

Figure 3. Schematic drawing representing simple double row rotator cuff repair technique (axial view).

Rehabilitation

The shoulder arm sling at the 30-degree abduction was routinely used for 3 weeks. The physical therapy protocol consisted of 4 phases. Postoperative rehabilitation was performed as two separate periods; clinic rehabilitation program and home exercise program. Patients were followed until assuring painless ROM and scapular dyskinesia.

Radiologic Evaluation

MRIs performed before and after the operation. Single radiologist who did not know the clinical outcomes of the patients evaluated the MRIs. Tendon continuity or recurrent tears were assessed on T2-weighted coronal oblique and proton density-weighted images as well as short inversion recovery sequences according to defined MRI criteria. Accordingly, at least one of the T2-weighted or repressed fat sections was interpreted in favor of rotator cuff tendons or to obtain a fluid-like signal image in favor of a full-thickness recurrent tear.

Statistical Analysis

IBM SPSS (Statistical Package for Social Sciences for Windows, Version 21.0, Armonk, NY, IBM Corp.) package software were used for statistical analyses. The Kolmogorov-Smirnov test was used to determine population distributions. We used the Shapiro-Wilk test for normally distributed data and Levene's Test for homogeneity of the variances. We found it unconvenient to use the Student's t-test as a result of not finding an appropriate condition. P-values less than 0.05 were considered statistically significant.

Results

Of the 27 patients in the study; 13 had Belt and Suspender's technique (2 male, 11 female) (Group A), 14 had simple double-row repair technique (4 male, 10 female) (Group B). The mean age of the patients having Belt and Suspender's technique was 61.89 years, body mass index (BMI) 26.5 kg-m^2 and the mean follow-up period was 23 months (7-47 months). The mean age of the patients having simple double-row repair technique was 60.27 years; mean BMI 27.0 kg-m^2 and the mean follow-up period was 45 months (7-82 months) (Table 1).

There was no statistically significant difference between postoperative ASES scores, VAS scores, range of motions (ROM) and complications (Table 2, 3). In both groups, failure of healing was seen in one patient in each group (Table 2).

Table 1: Characteristics of the patients in Group A and Group B.

	Group A (Belt and Suspender's technique)	Group B (Double-row repair)
Average Age (Years)	61.89 ± 9.31(40-70)	60.27 ± 5.41 (40-70)
Sex (M/F)	11 female, 2 male	10 female, 4 male
Weight (kg)	76.4±19.4	78.6±14.9
BMI (kg/m²)	26.5±5.5	27.0±5.8

BMI: Body Mass Index

Table 2: Number of complications.

	Belt and Suspender's technique	Double-row repair technique
Rerupture/failure of healing	1	1
Stiffness	0	0
Implant failure	0	0
Reflex symphatic distrophy	0	0
Infection	0	0

Table 3. Comparison of groups in terms of functional outcomes.

	Group A	Group B	P
ASES score-preop	47 ± 10	50.8 ± 14	NS
ASES score-postop	94.5 ± 12	91.5 ± 15	NS
VAS score-preop	7.2 ± 2	7.2 ± 1.8	NS
VAS score-postop	0.6 ± 2	0.6 ± 1	NS
Forward flexion-preop	160 ± 10	160 ± 15	NS
Forward flexion-postop	170 ± 11	172 ± 9	NS
Abduction-preop	165 ± 10	165 ± 20	NS
Abduction-postop	170 ± 10	171 ± 1	NS

NS: non significant (p>0.05)

Discussion

Primary findings of the present study suggest that both techniques are safe and reliable for the treatment of patte class 2 rotator cuff tears. In this study, failure of healing was detected in two patients with MRI. Given the patient's examination and functional scores, there was no relationship between failure of healing/re-tear and clinical outcomes. The clinical improvement of the patients in this study can be explained by the mechanism

described by Loehr et al. If the infraspinatus is undamaged, the rotator cuff centering function is not impaired [12]. In the present study none of the patients had infection, stiffness, and implant failure or reflex symphatic dystrophy. Healing failure was observed in one patient in both groups; however, clinical outcomes and functional scores of these patients were excellent.

The tear in the rotator cuff leads to progressive and perhaps irreversible degenerative changes in the rotator cuff muscles. Atrophy and fatty degeneration can affect various clinical parameters such as prediction of repair results and strength. For this reason, it has been suggested that the rotator cuff repair should be done before these changes occur. Recently, studies have been done quantitatively evaluating rotator cuff muscles [13]. MRI is used more frequently in these studies [14]. Turkmen et al. showed that the shoulder muscle mass can be accurately calculated by MRI in a clinic study [15]. One of the factors affecting the results of rotator cuff repair is tear size. Early term results show that the tear size does not affect the results of the surgery. However in recently, relationship between tear size and surgical outcomes is expressed in the literature [16]. Demirhan and Esenyel reported that the main application areas of arthroscopic rotator cuff repairs of middle and small rotator cuff tears and partial tears are to be used more frequently in the future with improved techniques and learning process [17]. However, there is still no consensus on this issue. Gartsman have stated that the tear size is not a decisive factor in the repair of the arthroscopic rotator cuff [18]. Other factors affecting clinical outcomes are biceps pathologies and subacromial impigement [16]. In the present study, the groups were homogeneous in this respect.

The most important component of the shoulder joint which is the most mobile and difficult to stabilize is the rotator cuff. Tears of rotator cuff disturb the comfort of the patient and care should be taken to early diagnosis and treatment. For an appropriate assessment; physical examination, ROM and muscle strength assessment and MRI is essential. When additional pathology is considered, methods such as EMG should be consulted. Patients' pathologies, causes of disease, age, presence or absence of systemic diseases are very important prognostic factors. Conservative treatment methods (physical therapy, rehabilitation, medical treatment, local injections) should be tried thoroughly even if operation is considered in all patients [19]. The desired result in treatments is to achieve a painless life, good muscle strength and ROM.

We think that middle and small rotator cuff tears and partial tears are the main application areas of arthroscopic rotator cuff repair. In addition, in the elderly, when it is necessary to perform partial repair in large ruptures, it is possible to obtain successful results with debridement [20]. Arthroscopic rotator cuff repair, a developing technique, will be used more frequently in the future with improved techniques and learning process. In this study, using one knotted suture anchor and one knotless suture anchor in the two techniques created different configurations. A relatively inexpensive technique has been used compared to conventional techniques with two techniques.

This study has certain limitations, most of which are inherent to its retrospective design and relatively small sample size. Assessment of clinical outcomes was limited to chart documentation, which has a risk of bias as can be expected in a retrospective study of this nature. This study has the strength of including two different techniques. The clinical relevance of this study is that surgeons may prefer "Belt and Suspender's Technique" in the surgical treatment of Patte class 2 chronic rotator cuff tears.

In conclusion, we believe that treatment for chronic rotator cuff tear using a Belt and Suspender's technique is as effective and reliable as simple double row cuff repair technique method with low complication rates and good results can be achieved in clinical outcomes in the early postoperative period.

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Factors related to recurrence in surgical treatment of hydatid cyst

Kist hidatiğin cerrahi tedavisinde rekürrens ile ilişkili faktörler

Ayetullah Temiz [1], Gürkan Öztürk [2], Abdullah Kısaoğlu [3], Ercan Korkut [2], Erdem Karadeniz [2]

Abstract

Aim: Curative treatment of cyst hydatid is surgical or interventional. However, recurrence is a very important problem today. In our study, we aimed to identify factors affecting recurrence in the surgical treatment of hydatid cyst.

Methods: This study was carried out retrospectively, and the files of 228 patients operated due to hydatid cyst were examined and data were recorded. 72 patients who did not meet the inclusion criteria were excluded from the study. Our results and factors affecting recurrence were examined.

Results: The recurrence was observed in 14 patients (8.9%). No relationship could be found between the recurrence and other factors such as the localization of the cyst, Gharbi classification, applied surgical technique, the contents of the cyst, treatment, and relationship between the cyst and bile tract, postoperative complications, the number and diameter of the cyst.

Conclusion: Prospectively planned and involving larger patient groups studies are needed for the determination of the factors affecting recurrence after surgery of liver hydatid cyst.

Key Words: Liver, hydatid cyst, surgery, recurrence

Öz

Amaç: Kist hidatiğin küratif tedavisi cerrahi veya girişimseldir. Bununla birlikte kist hidatik tedavisinde rekürrens çok önemli bir sorun olarak karşımıza çıkmaktadır. Biz çalışmamızda kliniğimizde hidatik kist nedeniyle opere edilen hastalarda rekürrensi ve rekürrense etki eden faktörleri belirlemeyi amaçladık.

Yöntemler: Retrospektif olan bu çalışmada, hidatik kist hastalığı nedeniyle cerrahi uygulanan 228 hasta dosyası incelenerek veriler kayıt altına alındı. Bu hastalardan çalışmaya dahil edilme kriterlerine uymayan 72 hasta çalışma dışı tutuldu. Sonuçlarımız incelenerek rekürrense etkili faktörler irdelendi.

Bulgular: Hastaların 14'ünde (%8.9) rekürrens tespit edildi. Kistin lokalizasyonu, Gharbi sınıflandırması, uygulanan cerrahi teknik, kistin içeriği, safra yolu iştiraki ve tedavisi, postoperatif komplikasyonlar, kistin sayısı ve çapı ile nüks arasında bir ilişki tespit edilmedi.

Sonuç: Karaciğer kist hidatiğinde cerrahi sonrası nükse etkili faktörlerin tespiti hususunda ileriye dönük olarak planlanmış ve daha geniş hasta gruplarını içeren çalışmaların yapılmasına ihtiyaç vardır.

Anahtar Kelimeler: Karaciğer, kist hidatik, cerrahi, rekürrens.

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Introduction

Hydatid cyst, or echinococcosis, is a zoonotic disease seen in parts of the world where breeding sheep is common. This is an important health care issue in our country, especially in Eastern and Southeastern Anatolian Regions [1, 2]. Hydatid cyst of the liver is asymptomatic in 40-60 % of the patients and pain is the most common symptom when it is symptomatic. Other common findings of this disease are jaundice and fever [2].

Diagnosis has become easier with improvement of imaging techniques; however, effective medical treatment is not still available. Curative treatment of hydatid cyst is surgical or interventional. Recurrence is a major problem following the surgical treatment of hydatid cyst. Despite the fact that it is a benign disease, treatment of its complications and recurrences are very challenging issues.

Case series reported from Turkey which is an endemic country for the disease have shown that recurrence rate is 3.4-24 % varying with different surgical methods [3]. In literature, recurrence rate during 5-year follow-up is determined as from 0.9 to11.3 % [4, 5].

We aimed to determine recurrence and factors affecting recurrence in patients who were operated in our clinic for hydatid cysts.

Material and methods

In this retrospective study, we examined medical records of 228 patients who were operated for hydatid cyst in our clinic between January 1999 and December 2008 after obtaining ethical committee approval. Content of this study was explained to all patients and informed consent forms were obtained. Data such as age, sex, complaints upon arrival, laboratory findings, and location of the cysts, diameter (cm), number and type of the cysts according to Gharbi classification were recorded as in previous studies [6]. Whether preoperative endoscopic retrograde cholangiopancreatography (ERCP) is performed, surgical methods, contents of the cyst, whether the cyst is associated with bile ducts, preoperative and postoperative albendazole use, complications and treatment for these complications were also recorded.

Patients with missing data in files, who cannot be reached and patients who did not accept to participate in the study were excluded from the study.

Seventy two patients who did not meet study criteria were excluded. Patients with complete files were reached and invited for control examination. Therefore, a total of 156 patients were included in the study. Abdominal ultrasound (US) and computed tomography (CT) in suspicious cases were performed. If recurrence was suspected with radiological studies, serologic tests with Enzyme-Linked Immunosorbent Assay (ELISA) were ordered. Live cysts detected in radiological studies in location of the primary disease or in any other intraabdominal region after treatment for a primary disease, and any increase in levels of serological tests was considered as diagnostic criteria for recurrence. When recurrence was detected in the primary disease site, it was referred to as local recurrence, and when it was found away from the primary site, it was referred to as disseminated disease.

Surgical procedures included both radical and conservative surgery. Radical surgery involved pericystectomy or hepatectomy (segmentectomy and lobectomy). Parenchymal transection was performed using the clamping technique or by ultrasonic aspiration (Cavitron Ultrasound Surgical Aspirator, CUSA). Vascular and biliary structures were connected using sutures and clips. Drainage with omentoplasty or capitonnage was performed in case of conservative surgery. Hypertonic saline at a concentration of 30% was used as a scolicidal solution. When cyst fluid containing bile was detected in the puncture, no scolicidal agent was used. To prevent accidental spillage of the cystic contents, the puncture site was covered with hypertonic saline solution soaked in a gauze. Small bile leaks in the cyst cavity were repaired by a primer suture. T tube was applied as an additional treatment of the primer for very large leaks.

Albendazole (Andazol, 200 mg oral tablet, Biofarma, Istanbul, Turkey) in a dose of 10 mg/kg/day was given twice daily for 15 days before the operation and 3 months after the operation as medical treatment.

In the follow-up period, an initial control abdominal ultrasound was routinely performed at the postoperative third month and the sixth month, followed by an annual examination.

Statistical analysis

Recorded data was analyzed with SPSS 15.0 for Windows Evaluation version (SPSS Inc., Chicago, IL, USA) by using T-test and chi square test; recurrence rates and factors affecting the recurrence were examined. Continuous variables are expressed as mean ± standard derivations (SD) and categorical variables as frequencies (percentages). p values smaller than 0.05 were interpreted as statistically significant.

Results

One hundred and fifty six patients were included in this study. Fifty nine patients (38%) were male and 97 patients (62%) were female. There were 14 recurrent cases (8.9%). Characteristic features of these 14 recurrence cases are shown in Table 1 and Table 2.

Complaints upon arrival were abdominal pain in all of the 156 patients, sensation of abdominal fullness in 23 (14.7 %) patients, jaundice and pruritus in 11 patients (7%), acute abdominal findings in two patients (1.2 %). All recurrent cases presented with abdominal pain but did not have other complaints. There was no relation between the complaints and recurrence (p=0.292). Demographic and clinical findings of the patients with and without recurrence are given in Table 3. Mean age was 44 ± 15.2 years in patients with recurrence and was 39.94 ± 17.55 years in patients without recurrence. There was no significant difference in demographic and clinical findings except the use of medical treatment between the patients with and without recurrence (Table 3). It was found that all patients with recurrence used both preoperative and postoperative albendazole treatment. However, for the patients without recurrence, it was detected that 115 patients (80%) and 125 patients (88%) used preoperative and postoperative medical treatment, respectively. However, the difference is not statistically significant (p=0.357).

Table 1: Characteristics of the patients with recurrence.

No	Sex (M/F)	Age (year)	Preop ERCP	Surgery	Preop Med. Tx.	Postop Med. Tx.
1	F	48	-	O+T	+	+
2	F	55	-	T	+	+
3	M	53	-	T	+	+
4	F	22	-	O+T	+	+
5	F	47	-	T	+	+
6	M	44	-	C+T	+	+
7	F	33	-	O+T	+	+
8	F	30	-	O+T	+	+
9	F	40	-	O+T	+	+
10	M	80	+	O+T	+	+
11	M	51	-	O+T	+	+
12	F	35	-	O+T	+	+
13	F	55	-	O+T	+	+
14	F	23	-	O+T	+	+

M: male, F: female, ERCP: endoscopic retrograde cholangiopancreatography, O: omentoplasty, T: tube drainage, C: capitonnage.

Table 2: Characteristics of the cysts of the patients with recurrence.

No	Localization[¥]	Cyst (n)	Diameter (cm)	Gharbi grade	Content	Biliary spillage	Biliary fistula
1	Liver (6-8)	1	15	2	Rock water	-	-
2	Pelvis	1	10	3	Rock water	-	-
3	Liver (6-7)	1	10	3	Purulent	-	-
4	Liver (7-8)	1	10	3	Rock water	-	-
5	Liver (7)	1	10	3	Rock water	-	-
6	Liver (8)-uterus	2	10	4	Rock water	-	-
7	Liver (6-7)	1	10	4	Rock water	-	-
8	Liver (7)-pancreas	2	10	4	Rock water	-	-
9	Liver (6-7)	1	10	3	Purulent	-	-
10	Liver (6-8)	4	15	3	Bile	+	+
11	Liver (4-6)	2	15	3	Rock water	-	-
12	Liver (7-8)	1	10	3	Rock water	-	-
13	Liver (7-8)	1	5	4	Rock water	-	-
14	Liver (8)	1	13	4	Rock water	-	-

[¥]: Numbers in parenthesis showing number of the segment/segments of the liver.

Discussion

Different recurrence rates of hydatid cyst disease have been reported in different series. The recurrence rate during a 5-year follow-up period has been reported to be 0.9%–11.3% in the literature [3, 4]. Recurrence rate can differ in the same study center at different time points. Secchi et al. [4] had reported a recurrence rate of 7.2 % in the early postoperative stage of conservative surgery before 1990, whereas this rate was found to decrease to 1.6 % for the same type of surgery after 1990. Advancements in techniques and experience could have played a major role in this decrease. Our rate was consistent with the literature.

It is suggested that follow-up of the operated patients every 6 months by performing an annual abdominal ultrasound examination for at least 3 years is essential because most recurrences have been observed in this time period as in the present study [7].

There are no prospective studies determining the reasons for recurrence in hydatid cyst disease. However, some important aspects have been emphasized in relation to recurrence. The primary reasons that have been emphasized in the literature [8-10] are as follows:

a) Spillage of cyst contents into the intraperitoneal area during discharge of cyst,
b) Improper use of scolicidal agents,
c) Undiagnosed cysts before and after surgery,
d) Failure to remove all live cyst contents during the first surgery,
e) Failure to reach pockets in the pericyst during the conservative approach,
f) Direct rupture of the cyst into the liver.

Maliki et al. [11] stated that cysts localized in central segments have statistically significantly higher recurrence rates than those localized in lateral segments. However, two studies from our country could not find any statistically significant effect of cyst localization on recurrence [12]. We also did not find any significant relationship between cyst localization and recurrence. The literature states that there is no relationship between Gharbi classification and recurrence [11,12]. Similarly, in our study, despite the fact that the cysts were primarily of Gharbi classification type 3, there was no relationship with recurrence.

In the literature, it has been reported that the size and number of cysts have an effect on recurrence. A study involving 672 patients reported that when there are three or more cysts, the recurrence rate increases by 3.8 times. The same study demonstrated that the recurrence rate is higher in cysts measuring 10 cm or larger than in cysts measuring smaller than 10 cm and this difference was found to be statistically significant in the single variant analysis [11]. Nonetheless, there are studies that report that cyst size and number have no impact on recurrence rate [12]. Similarly, in this study, we determined that cyst size and number do not have any effect on recurrence.

The World Health Organization (WHO) reports that preoperative medical treatment with albendazole decreases recurrence and facilitates surgery by reducing the pressure inside the cyst, thus recommending the use of albendazole [13,14]. In a study conducted by Arif et al. [15], the recurrence rate in patients who did not use albendazole was 18.5%, whereas it was 4.16% in patients who used albendazole. However, in our study, all patients with recurrence used albendazole in both the preoperative and postoperative periods, but among those patients who did not have recurrence, 115 (80 %) patients used it preoperatively and 125 (88 %) used it postoperatively. However, the variety of albendazole use has not reached a statistically significant level among the groups.

One of the major reasons for recurrences, primarily local recurrences, is insufficient or inappropriate surgical technique. Local recurrence risk is lower in radical surgical interventions such as pericystectomy and liver resection, because these methods clear the cyst completely without any remains. It has been argued in the literature that with methods that leave some or all of the pericystic tissue, the recurrence rates will be higher [16]. This is based on the view that live scolex can remain in the pericystic tissue.

On the other hand, there are studies showing that scolexes are not found in the pericystic tissue histologically [9]. Thus, the view that defends recurrences occurs because the remaining tissue is weakened. A general idea is that recurrence rates with radical surgery are lower than the rates with conservative surgery. A study from our country stated that recurrence rates with conservative surgery performed by experienced surgeons decreased from 20% to 5% [17]. Secchi et al. [4] reported that the recurrence rate after radical surgery was 1.3% in a multicenter study of 1412 cases. Aydin et al. [3] conducted a study in which they performed radical surgery in 92 patients and conservative surgery in 129 patients and reported the recurrence rates as 3.2 % and 24 %, respectively.

Another study reported a recurrence rate of 7.9 % in patients who underwent radical surgery during a 10-year follow-up [11]. Based on these results, it can be stated that radical surgical methods that are performed without opening the cysts can decrease the recurrence risk but do not eliminate it.

In our study, there were no recurrences in patients who underwent radical surgery; on the other hand, 14 recurrence cases were detected among patients who underwent conservative surgery. There was no statistically significant effect of the surgical method on the recurrence rate. Nevertheless, it is expected that radical surgery has a statistically significantly lower recurrence rate. However, due to the small number of patients who underwent radical surgery, it was difficult to interpret this result.

In our clinic, conservative interventions have been implemented for a longer period of time and in a more standardized manner. Considering that conservative interventions are much common and radical surgical interventions are rarely performed, a recurrence rate of 8.9% is notably low for our clinic. Moreover, the reported rates have been almost 20 % in the majority of the studies.

Table 3. Distribution of the features of the cyst between the groups.

Variable		Recurrence (n(%))		
		Yes (n=14)	No (n=142)	p
Age (year)		44.00±15.2	39.94±17.55	0.205
Localization	Liver right lobe	9 (64.28)	78 (54.92)	0.292
	Liver left lobe	1 (7.14)	27 (19.01)	
	Liver right+left lobe	1 (7.14)	18 (12.67)	
	Liver + abdomen	2 (14.28)	13 (9.15)	
	Abdomen	1 (7.14)	6 (4.22)	
Number of cysts		1.43±0.93	2.54±8.49	0.465
Cyst diameter (cm)		10.93±2.73	11.63±4.91	0.475
Gharbi grade	Type 1	0	0	0.679
	Type 2	1 (7.14)	11 (7.74)	
	Type 3	9 (64.28)	106 (74.64)	
	Type 4	4 (28.57)	23 (16.19)	
	Type 5	0	2 (1.40)	
Preoperative ERCP	Yes	1 (7.14)	15 (10.56)	0.687
	No	13 (92.85)	127 (89.44)	
Surgical technique	Omentoplasty	0	2 (1.40)	0.170
	Capitonnage	0	2 (1.40)	
	Tube drainage	3 (21.42)	16 11.26)	
	Omentoplasty+ tube drainage	10 (71.42)	101 (71.12)	
	Capitonnage+ tube drainage	1 (7.14)	4 (2.81)	
	Splenectomy	0	5 (3.52)	
	Pericystectomy	0	11 (7.74)	
	Resection	0	1 (0.70)	
Cyst contents	Rock water	11 (78.57)	88 (61.97)	0.465
	Purulent	2 (14.28)	28 (19.71)	
	Bile	1 (7.14)	26 (18.30)	
Biliary tract subsidiary	Yes	1 (7.14)	33 (23.24)	0.563
	No	13 (92.85)	109 (76.76)	
Treatment of biliary tract subsidiary	Primary suture	1 (7.14)	25 (17.61)	0.550
	T-tube	0	3 (2.11)	
	Choledochoduodenostomy	0	5 (3.52)	
Postoperative complications	Wound infection	1 (7.14)	7 (4.92)	0.267
	Biliary fistula	1 (7.14)	8 (5.63)	
	Cholangitis	0	2 (1.40)	
	Biloma	0	3 (2.11)	
Use of medical treatment				0.357
	Preoperative	14 (100)	115 (80)	
	Postoperative	14 (100)	125 (88)	

There is a lack of sufficient studies in the literature analyzing the relationship between the content of the cyst and the recurrence rate. A study comprising 63 cases reported that 3% of recurrence cases had bile duct involvement, and the relationship between bile duct involvement and recurrence was statistically non-significant [12]. In our study, 7% of recurrence cases had bile duct involvement, but the relationship was not statistically significant.

Postoperative recurrence rate is parallel to the length of follow-up. Studies have reported that the recurrence rate after 1 year of surgery was 2.3% and 9.1% after 10 years. Therefore, an extended length of follow-up is recommended. Hydatid cyst disease that has recurred generally exhibits its symptoms 3–4 years after surgery [12, 18, 19]. That is why it is recommended to perform USG once a year and a CT scan biannually for 5 years after surgery [11].

There is no study in the literature regarding the relationship between preoperative ERCP and recurrence. In our study also, we did not determine any significant relationship between preoperative ERCP and recurrence rate. There was one patient with recurrence, and a total of 16 patients had preoperative ERCP. These numbers are inadequate in this regard, which could be the reason for this result. We believe that prospective studies with larger sample sizes are necessary in this area.

In conclusion, hydatid cyst is a benign disease and treatment choices for this disease should be made accordingly. We consider that in experienced clinics, the choice of treatment should be conservative and radical interventions should be limited to appropriate cases. Prevention of contamination during surgery and meticulousness in cavity management increase the value of these surgical interventions. Studies that are prospectively planned and involving larger patient groups are needed for determining the factors affecting recurrence after surgery of the liver hydatid cyst.

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Hesperidin triggering apoptosis on neuroblastoma cell

Nöroblastoma hücrelerinde hesperidinin apoptozu tetiklemesi

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Abstract

Aim: Neuroblastoma accounts for approximately 10% of all pediatric cancers and approximately %15 of cancer related deaths in children. Understanding of the molecular mechanisms which play role in the progress of this cancer type may lead to develop more effective strategies for therapy. Flavanoids are popular subject for this new strategies. Hesperidin is herbal flavonoid which is found abundantly in citrus that has been studied on several cancer cell lines. For this purpose, it was aimed to investigate is the apoptotic effects of hesperidin on neuroblastoma tumors using SH-SY5Y cell line.

Methods: Hesperidin was performed on SH-SY5Y and MRC-5 cell line by WST-1, Caspase-3 and Annexin V in a time and dose dependent manner.

Results: 2.5 µM hesperidin and 5 µM hesperidin were found the most suitable dosage for neuroblastoma cell line because of the success on decreasing cell proliferation. Hesperidin has resulted with the ability for apoptotic cell death compared with control group [MRC-5 cell line, $p<0.05$ for all]. 2.5 µM and 5 µM hesperidin concentration for 48h were ended up early apoptotic results as 53.65% for 2.5 µM and 38.90% for 5 µM. There was no significant change on caspase-3 activity.

Conclusions: Our study suggests that hesperidin would be effective against neuroblastoma tumors. We believe with further investigation this study will be helpful for developing new research areas in neuroblastoma tumors.

Keywords: Hesperidin, neuroblastoma, apoptosis.

Öz

Amaç: Çocukluk çağında rastlanan pediatrik kanserlerin yaklaşık %10'luk bir kısmını oluşturan nöroblastoma, çocuklarda kansere bağlı ölümlerin yaklaşık %15'lik bir bölümünden de sorumludur. Bu kanser türünün ilerlemesi esnasında rol alan moleküler olayların anlaşılması, tedavi açısından daha etkili yöntemlerin ortaya konmasına aracılık edebilecektir. Flavanoidler kanser üzerinde yeni tedavi stratejileri geliştirilmesi açısından popülerdir. Hesperidin turunçgillerde bolca bulunan ve bir çok kanserli hücre hattında çalışılan bir flavonoiddir. Çalışmamızda hesperidinin nöroblastoma hücre hattı SH-SY5Y üzerinde apoptotik etkinliğinin araştırılması amaçlanmıştır.

Yöntem: Hesperidinin SH-SY5Y hücre proliferasyonu ve canlılığı üzerinde doz bağımlı etkisi için WST-1, kaspaz enzim aktivitesinin değerlendirilmesi için Kaspaz 3/BCA ve apoptotik değerlendirme için Annexin V analizleri yapılmıştır.

Bulgular: 2,5 µM ve 5 µM hesperidin hücre proliferasyonu üzerinde azalma etkisi yarattığından hücre hattında uygulanmak üzere seçilmiştir. Hesperidin kontrol grubuna göre nöroblastoma üzerinde apoptotik hücre ölümüne neden olmuştur [MRC-5 hücre hattı, hepsi için $p<0.05$]. 2,5 µM hesperidin ve 5 µM hesperidin 48 saatlik inkübasyon sonucu sırası ile %53,65 ve %38,90 apoptoz ile sonuçlanmıştır. Kaspaz 3 aktivitesinde ise herhangi bir değişim gözlenmemiştir.

Sonuç: Bu çalışmamızda elde ettiğimiz sonuçlardan, nöroblastoma tümörlerinde hesperidinin antikarsinojenik etki gösterdiği izlenimi edinilmiştir. Elde edilen verilerden yola çıkarak, çalışmamızın yapılacak ileri araştırmalar ile birlikte nöroblastoma tümörlerinde yeni araştırma alanlarının oluşmasında katkısı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: nöroblastoma, hesperidin, apoptoz.

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Introduction

Neuroblastoma is a rare type of cancer even though it is very common extracranial tumor in childhood. The tumors are generally composed of sympathetic nervous system and originated from the adrenal glands; however, the abdomen, the chest, the spinal cord and the paraspinal ganglia might be the origin [1].

Approximately 650 new cases of neuroblastoma are diagnosed each year in United States. Almost 90% of these cases are up to 5 years old and also diagnosed age average is 19 months old [2]. Patients are divided to low, medium and high-risk groups according to their clinical, pathological conditions and genetic factors. Survival rates of the low risk group are up to 90%. Avoidance of aggressive treatment and minimization of chemotherapy-induced toxicity is the main goal for all cases except in the high-risk group [3]. In almost 70% of the patients, metastasis can be seen after that they diagnosed. In this way, these groups of the patients are changing into high-risk group. The predicted 5-year survival rate of high-risk group of neuroblastoma patients is between 40%-50%. However, almost half of these patients are relapsing, by that way it makes the 5-year survival rate decrease to the range of 20% [4, 5].

Aggressive approaches such as chemotherapy, surgical intervention, autologous hematopoietic stem cell [autologous-HSCN] and radiation therapy are followed in the treatment of neuroblastoma for the high-risk group. After success of the treatment, the aim is to prevent the recurrence of the disease [4, 6].

Even though the choices of treatment options are still going on, the new approaches should be improved for high-risk neuroblastoma patients. The main reason of the treatments are not sufficient is because of the resistance to chemotherapy [7]. That is why new, comprehensive and effective treatment options should be investigated [8].

Hesperidin is a flavan-on glycoside that founds in citrus such as oranges, lemon [9]. Some studies have shown that hesperidin has the ability to protect system from oxidative damage and free radicals [10]. In addition to this, hesperidin has also the several properties about anti-inflammatory, analgesic, antifungal, antiviral, and anticancer effects [11, 12].

In this research, we aimed to investigate of hesperidin role on SH-SY5Y neuroblastoma cell line due to its possible capability of additional cancer treatment.

Material and methods

Cell culture method

Neuroblastoma cell line SH-SY5Y and MRC-5 cell line as a healthy control was obtained from ATCC [American Type Culture Collection, Manassas, VA]. SH-SY5Y neuroblastoma cell line was cultured in a mixture media containing EMEM [ATCC, 30-2003, L-Glutamin] and Ham's F-12 [Lonza, BE12-615F, L-Glutamin]. MRC-5 cultured with RPMI 1640 [Lonza, BE12-918F, L-Glutamin]. All media was prepared with 1% penicillin/streptomycin and 10% fetal bovine serum. Cells were cultured in a condition of 37°C in 5% CO_2.

Level of cytotoxicity

WST-1 cell proliferation assay was used to determine the cytotoxicity level of hesperidin on SH-SY5Y and MRC-5 cell lines. $1x10^4$ cells/well was seeded for treatment. 0, 2.5, 5, 10, 25 and 50 µM concentrations of hesperidin were treated and incubated for 24, 48 and 72h 37ºC in 5% CO_2 in time and dose depended manner on cells including MRC-5 cell line. After the incubation period, 10 µl of WST-1 were added all wells for 2h. Color development was measured at 450 nm using a Multiscan ELISA reader [Thermo Fisher Scientific, Germany].

Detection of caspase-3 enzyme activity

One of the important sign for apoptosis is cellular caspase-3 enzyme activity. Caspase-3 activity was determined by caspase-3 colorimetric assay kit [BioVision Research Products,USA]. The protocol was used step by step according to kit instruction. The aim of the procedure is to measure chromophore p-nitroanilide [pNA] after cleavage from the labeled substrate DEVD-pNA by spectrophotometry under 405 wavelengths on ELISA reader [Thermo Electron Corporation Multiskan Spectrum, Finland]. In addition, Bradford assay was used to normalize the protein concentrations.

Analysis of phosphatidylserine exposure by Annexin V

When apoptosis occurs in the cell, phosphatidylserine [PS] components start to move from cell through the cell surface. This make PS work as an apoptotic marker. Due to the PS role as a marker, the level of PS was examined by staining with the green fluorescent Annexing V-FITC [BD Pharmingen, Germany]. First of all, cells were suspended with dyes in 250µl buffer. After suspension cell mixture were analyzed immediately by flow cytometry.

Statistical analysis

Results are expressed as the mean standard error of the mean [SEM]. The data were analyzed using one-way ANOVA.

Results

Hesperidin inhibiton of SH-SY5Y cell line in a time and dose manner

0, 2.5, 5, 10, 25, 50 µM concentrations of hesperidin were used to determine of cytotoxicity level for 24, 48 and 72h. Results are given on Figure 1. 2.5 µM and 5 µM hesperidin concentrations were the most effective for decreasing the proliferation with the time of 48h (p=0.008) for SH-SY5Y cell line. Moreover, there were no important changes in the healthy cell line (p=0.014). The only loss of viability values were 3.97% for 2.5 µM hesperidin, and 2.93% for 5 µM hesperidin were found for MRC-5 cell line as healthy control.

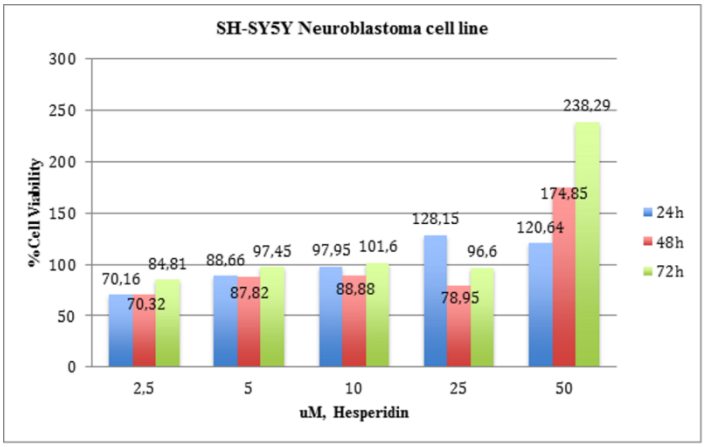

Figure 1. Cell viability after hesperidin treatment.

Detection of caspase-3 enzyme activity

SH-SY5Y and MRC-5 cell lines were incubated with 2.5 µM and 5 µM hesperidin concentrations for 48h according to

the cytotoxicity test. Compared with untreated cells, there was almost no change in any manner (Figure 2). There was also no change in caspase3 activity of MRC-5 cells under the same conditions.

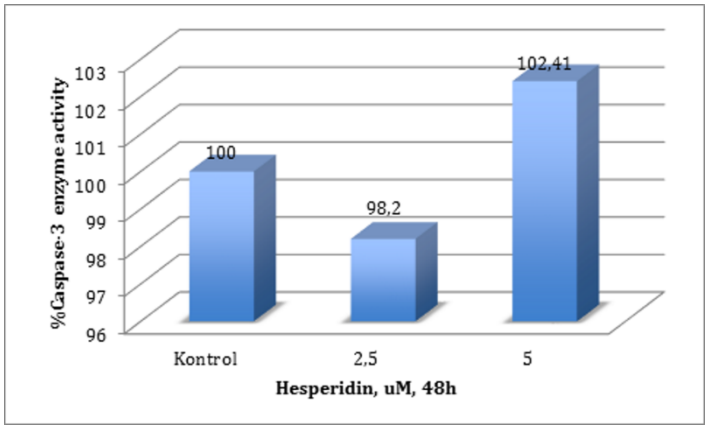

Figure 2. Caspase-3 enzyme activity for SH-SY5Y.

Phosphatidylserine exposure

Annexing V was performed SH-SY5Y and MRC-5 cell lines that both were exposed with 2.5 µM and 5 µM hesperidin concentration for 48h. According to results, hesperidin has the ability for apoptotic cell death compared with control group (MRC-5 cell line, $p<0.05$ for all). Furthermore, there were no important changes in MRC-5 cell line (Figure 3).

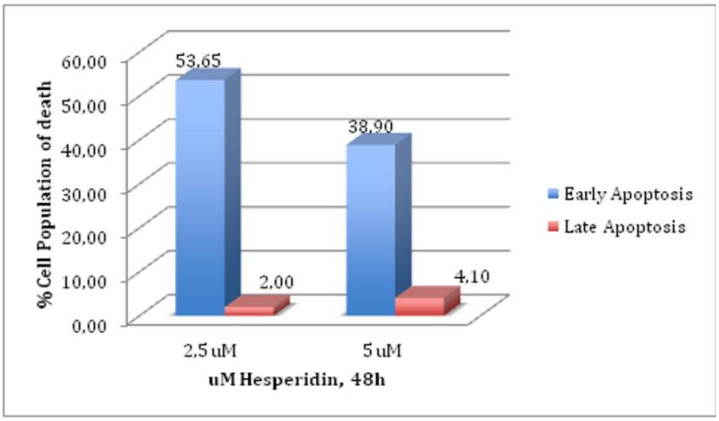

Figure 3. Cell population of death for SH-SY5Y

Discussion

Neuroblastoma treatment has some difficulties with resistance to chemotherapeutic agents especially if the patients are in high-risk group. The survival rate, resistance capacity and relapsing the disease are important points for improving new options or additional treatments [7, 8]. Flavonoids that found in citrus are secondary metabolites with several anti-oxidative, anti-inflammatory, anti-carcinogenic and neuroprotective capacity [13]. Due to the previous studies, even high concentration of hesperidin could not be a reason for any serious side effects [14]. According to literature, we could not be able to find any study about hesperidin and neuroblastoma relation. We have worked with SH-SY5Y cell line for neuroblastoma and MRC-5 as a healthy cell line. Hesperidin works as an anti-carcinogenic effect by stopping the cell cycle [15] and stimulating the apoptotic pathways [16]. Ghorbani et al. [17] have worked with hesperidin activity on NALM-6 leukemia cell line and their study has resulted with apoptosis and also stimulation to increased level of p53 and down regulation of NF-kB. Febriansah et al. [18] have investigated to time and dose depended hesperidin concentration on human breast cancer cell line. Not only they have come up with hesperidin's apoptotic potential, but also hesperidin has the antagonistic effect with doxorubicin. Yumnam et al. [19] have showed the inhibition of proliferation on hepatocellular carcinoma cell line by using hesperidin however they also have resulted that there was no change on healthy cell line. Tamilselvam et al. [20] have pointed out that only hesperidin could not have any change on proliferation of SK-N-SH neuroblastoma cell line; however, hesperidin and rotenone had the effective results by decreasing the proliferation by using at the same time. The study has showed that rotenone has the effective role by increasing the expression of caspase-3 and caspase-9. They also claim that only hesperidin has not been effective on any caspase activity. In addition, the research has mentioned about neuroprotective activity of hesperidin on neuroblastoma cell line [20]. In our study, we have the similar caspase activity results on SH-SY5Y neuroblastoma cell line by using only hesperidin. Dourado et al. [21] have showed the apoptotic activity of hesperidin on Loucy leukemia cell line by using Annexin V-FITC method. Their results have showed that 80% survival cell, 14% early apoptotic cell and 5% late apoptotic cell by using 10 uM hesperidin. Not only our results have the similar apoptotic effect with Dourado et al. [21] on SH-SY5Y cell line but also we have seen no change on MRC-5 cell line that was our healthy control. Hesperidin might be effective for apoptosis with another caspase enzyme activity except caspase-3.

In conclusion, hesperidin has shown anticarcinogenic activity on neuroblastoma cells consistent with other investigations of the mechanism of inducing hesperidin in our study.

We think that it will be an important step in eliciting details of the induction mechanism of hesperidin and in terms of target-oriented therapy for neuroblastoma treatment with further studies and additional methods.

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Arch Clin Exp Med 2018;3(3):127-131. e-ISSN: 2564-6567

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The impact of vitamin D on rheumatoid arthritis: real or just patient's perception?

Vitamin D'nin romatoid artrit üzerindeki etkisi: gerçek mi yoksa sadece hastanın algısı mı?

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Abstract

Aim: In this study, our aims were to identify vitamin D levels in rheumatoid arthritis (RA) individuals as compared to controls and the impact of vitamin D levels on both objective and subjective assessments in RA patients.

Methods: The current study was a prospective case-control study conducted on 108 RA patients and 50 age-gender matched healthy controls. We first compared the levels of vitamin D among the RA patients and controls. Demographic and clinical data, parameters of disease activity, inflammatory markers, rheumatoid factor and anti-cyclic citrullinated peptide seropositivity and radiological damage scores were recorded in RA patients. These patients were also demanded to complete RA Quality of Life Questionnaire (RAQoL), fatigue severity scale (FSS) and Health Assessment Questionnaire (HAQ).

Results: D vitamin levels in RA patients were significantly lower than healthy controls (p=0.001). Vitamin D deficiency was determined in 73% of the RA patients and 52% of the controls. Vitamin D deficiency was not associated with disease activity (p=0.862). There was no significant relationship among vitamin D levels and all subjective and objective assessments (p>0.05 for all).

Conclusion: Vitamin D deficiency was common in RA participants than normal population. However, it was not shown that there was a significant relationship between vitamin D levels and objective and subjective assessments of disease, including disease activity, inflammatory markers, rheumatoid factor and anti-cyclic citrullinated peptide seropositivity, radiological damage scores, RAQoL, FSS and HAQ.

Key Words: Rheumatoid Arthritis, Vitamin D, Radiological damage

Öz

Amaç: Bu çalışmadaki amaçlarımız, romatoid artrit (RA) hastalarında D vitamini düzeylerini ve D vitamini düzeylerinin objektif ve subjektif değerlendirmelere etkisini belirlemektir.

Yöntemler: Bu çalışma, 108 RA hastası ve yaş ve cinsiyete göre eşleştirilmiş 50 sağlıklı kontrol üzerinde yapılan bir prospektif olgu-kontrol çalışmasıdır. Öncelikle RA hastaları ve kontroller arasında D vitamini düzeylerini karşılaştırdık. RA hastalarının demografik ve klinik verileri, hastalık aktivitesi parametreleri, inflamatuvar belirteçleri, romatoid faktör ve anti-siklik sitrüllenmiş peptid seropozitifliği ve radyolojik hasar skorları kaydedildi. Hastalardan ayrıca RA Yaşam Kalitesi Anketi (Rheumatoid Arthritis Quality of Life, RAQoL), yorgunluk şiddet ölçeği (Fatigue Severity Scale, FSS) ve Sağlık Değerlendirme Anketi (Health Assessment Questionnaire, HAQ) tamamlamaları istendi.

Bulgular: RA hastalarında D vitamin düzeyleri sağlıklı kontrollerden anlamlı derecede düşüktü (p=0,001). RA hastalarının % 73'ünde ve kontrollerin % 52'sinde D vitamini eksikliği tespit edildi. D vitamini eksikliği, hastalık aktivitesi ile ilişkili değildi (p=0,862). D vitamini seviyeleri ile tüm subjektif ve objektif değerlendirmeler arasında anlamlı bir ilişki bulunamadı (hepsi için p>0,050).

Sonuç: D vitamini eksikliği RA hastalarında normal popülasyona göre daha sık görülmektedir. Ancak, vitamin D düzeyleri ile hastalık aktivitesi, inflamatuvar belirteçler, romatoid faktör ve anti-siklik sitrüllenmiş peptid seropozitifliği, radyolojik hasar skorları ve RAQoL, FSS, HAQ ile ilişkili hastalığın objektif ve subjektif değerlendirmeleri arasında anlamlı bir ilişki bulunduğu gösterilemedi.

Anahtar kelimeler: Romatoid Artrit, D vitamini, Radyolojik hasar

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Introduction

Vitamin D is a fat-soluble seco-steroid hormone that is synthesized naturally in the human skin by sunlight. In the liver, it is transformed to 25-hydroxyvitamin D [25 (OH)D], which is the most important metabolite for determining the capacity of vitamin D in the body. Thereafter, it is transformed into the biologically active form [1, 25 (OH)2D] [1]. It has been shown that vitamin D takes an important part not only in bone metabolism, but also in the immune system [2, 3].

Rheumatoid arthritis (RA) is a widespread, chronic inflammatory disorder (affecting almost 1% of the world population) characterized by progressive joint destruction and various systemic involvements. Although its etiopathogenesis is not completely understood, various genetic and non-genetic factors have been responsible for RA. It was shown that inadequate vitamin D levels might be an environmental trigger of RA [4]. Another interesting issue is whether vitamin D has an inverse relation with RA activity. The evidence from various studies concerning the link between serum vitamin D concentration and disease activity is inconsistent [5-7]. Some studies also revealed that the impact of inadequate vitamin D levels on disease activity in the RA individuals was associated with more subjective variables (eg, Visual Analog Scale (VAS) pain, tender joint count (TJC)) than a real objective immunomodulatory effect [8]. Both objective and subjective patient-based assessments are required to understand the potential association among RA and the level of vitamin D. Therefore, our aims were to identify vitamin D levels in RA individuals as compared to controls and the impact of vitamin D levels on both objective and subjective assessments in RA patients.

Material and methods

Our prospective case-control study was conducted in the Physical Medicine and Rehabilitation department from October 2017 to March 2018. The ethical approval of the present study (Ethics Committee of Ankara Numune Training and Research Hospital; Decision no/date: 2014-726/03-01-2014) was obtained. All participants gave informed consent to participate in the current study in accordance with the Helsinki Declaration.

Patients with RA based on European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) 2010 criteria [9], diagnosed at least one year, aged greater than 18 were included. Besides, age-gender matched healthy individuals from participating patients' relatives as control were included. Subjects were excluded if they had hyperparathyroidism, hyperthyroidism, malnutrition, renal and hepatic dysfunction, previous biologic therapy history and received Vitamin D supplementation or drugs which can affect Vitamin D metabolism (i.e. thyroxin, diuretics, and anticonvulsants) in the past 12 months.

Age (years), gender, body mass index (BMI) (kg/m2), waist circumference (cm), smoking status, duration of disease (months), the status of rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), morning stiffness time (minute), the patient's assessment of global well-being (10 cm VAS score), TJC (0-28), RA Quality of Life Questionnaire (RAQoL) (0-30) [10,11], fatigue severity scale (FSS) (0-7) [12], and Health Assessment Questionnaire (HAQ) (0-3) [13], swollen joint count (SJC) (0-28) were documented. Serologic evaluation including the serum concentration of 25(OH)D (ng/mL), erythrocyte sedimentation rate (ESR) (mm/h) , and C-reactive protein (CRP) (mg/dL) were analyzed. Finally, all x-ray examinations of the hands in posteroanterior view were estimated considering the van der Heijde modified Sharp score (vdHSS) [14].

Vitamin D level was evaluated with the blood test by the chemiluminescence method. Vitamin D status <20 ng/mL was determined as deficiency, vitamin D status < 10 ng/mL was determined as severe deficiency [15, 16]. We split 108 RA participants into two categories based on 25(OH) D levels-less than 20 ng/mL as cut-off value [17].

The disease activity was assessed using the Disease Activity Score 28-erythrocyte sedimentation rate (DAS28-ESR). All RA participants were categorized into four groups according to their DAS28-ESR: Remission (<2.6), low disease activity (2.6-3.2), moderate disease activity (>3.2–5.1) and high disease activity (DAS28>5.1) [18].

Statistical Analysis

Whole analyses were established using Statistical Package for the Social Sciences-21.0 (SPSS 21.0) software. Shapiro-Wilk test was utilized to test for normality; parametric and nonparametric tests were performed according to results. We used mean ± standard deviation (SD) for normally distributed variables (age, waist circumstance, BMI) and median, minimum-maximum for continuous variables which are not normally distributed. Categorical data are presented as percentages. Differences between patients and controls were checked using Mann-Whitney U test or independent samples T-test according to normality test. The homogeneity of the distributions of categorical variables was determined using chi-square tests. Spearman correlation coefficient was used and the correlation coefficient ranges in value from −1 to +1. P<0.050 was defined as statistically significant.

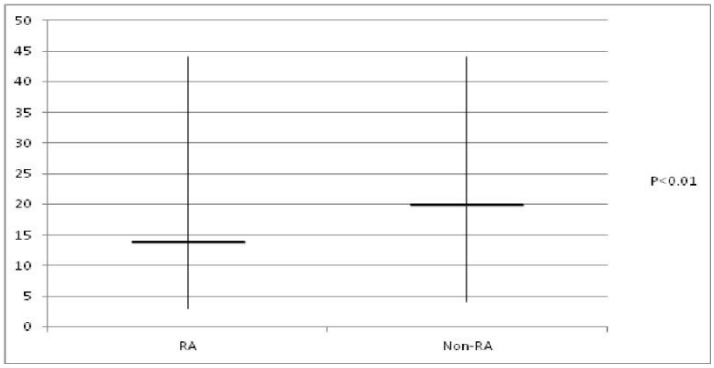
Results

The current study involved 108 participants with RA (female to male ratio: 3) and 50 healthy controls (female to male ratio: 4.56) whose mean ages were 52.66 ±12.23 years and 51.54±9.72 years, respectively. Demographic features of the individuals are summarized in Table 1. There was no significant difference for age, gender, BMI, and smoking status (Table 1), while serum vitamin D concentrations were significantly lower in RA subjects compared to the controls [14.69±9.90 ng/ml, 20.46±9.79 ng/ml, respectively; (p=0.001)] (Figure 1).

Table 1: Demographic and clinical features of RA and healthy controls.

	Patients with RA (n=108)	Control (n=50)	p
Age (years)$^{\mu}$	52.66±12.23	51.54±9.72	0.343
Female sex$^{\pi}$	81(75)	41(82)	0.329
BMI (kg/m^2)$^{\mu}$	28.61±5.86	26.82±5.86	0.064
Current smokers$^{\pi}$	44 (40.7)	20(40)	0.930
Vitamin D level (ng/ml)$^{\mu}$	14.69±9.90	20.46±9.79	0.001

$^{\mu}$: mean±standard deviation, $^{\pi}$: n (%)
RA: Rheumatoid arthritis, BMI: Body mass index.



RA: Rheumatoid arthritis
Figure 1: Vitamin D levels (ng/ml) (Y axis) in the RA and healthy controls.

Variables	All participants (n= 108)	Vitamin D <20 ng/mL (n= 79)	Vitamin D ≥20 ng/mL (n= 29)	P
Age (years)[μ, β]	52.66±12.23 54 (21-79)	51.84±12.16 53 (21-79)	54.89±12.36 56 (23-75)	0.177
Female sex[π]	81 (75)	60 (75.9)	21 (72.4)	0.707
BMI (kg/m^2)[μ, β]	28.61±5.86 28.54 (14.86-45.79)	28.82±5.63 28.72 (18.61-44.14)	28.04±6.53 27.10 (14.86-45.79)	0.390
Waist circumference, (cm)[μ, β]	96.10±14.82 97(58-136)	96.88±14.71 98(65-136)	93.96±15.15 95(58-132)	0.248
Current smokers[π]	44 (40.7)	34 (43)	10 (34.5)	0.423
Disease duration (months)[μ, β]	147±92 132 (12-468)	145±94 120 (12-468)	151±90 144 (12-420)	0.654
RF positivity[π]	80 (74.1)	62 (78.5)	18 (62.1)	0.085
CCP positivity[π]	80 (74.1)	59 (74.7)	21 (72.4)	0.811

Table 2: Comparison of all participants' descriptive data in respect to vitamin D status.
[μ]: mean±standard deviation, [π]: n (%), [β]: median (min-max)
BMI: Body mass index, RF: Rheumatoid factor, CCP: cyclic citrullinated peptide.

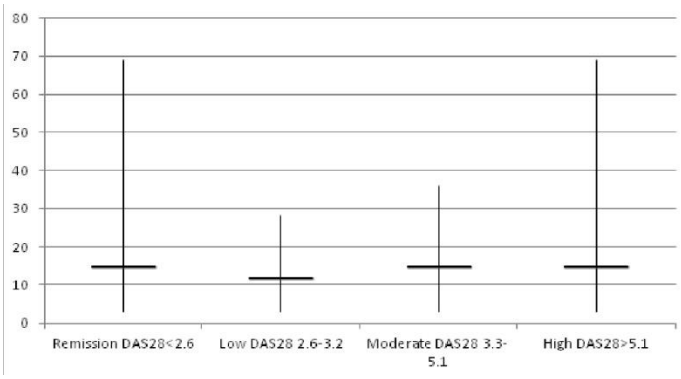
Variables	All participants (n=108)	Vitamin D <20 ng/mL (n=79)	Vitamin D ≥20 ng/mL (n=29)	P
Morning stiffness (minute)[μ, β]	39.72±66.74 5 (0-300)	36.51±63.56 5 (0-300)	48.44±75.26 15 (0-300)	0.472
VAS pain (0–10) [μ, β]	3.63±2.85 3 (0-9)	3.5±2.81 3 (0-8)	3.79±3.0 4 (0-9)	0.758
TJC[μ, β]	8.26±9.57 4 (0-28)	8.77±9.61 7 (0-28)	6.89±9.48 2 (0-28)	0.576
RAQoL[μ, β]	12.75±10.37 11 (0-30)	12.58±10.29 11 (0-30)	13.24±10.77 11 (0-30)	0.803
FSS[μ, β]	3.55±2.07 2.72 (1-6.78)	3.45±2.10 2.55 (1-6.78)	3.82±1.99 3.33 (1-6.78)	0.238
HAQ[μ, β]	0.96±0.80) 0.75 (0-2.75)	0.96±0.79 0.75 (0-2.75)	0.98±0.86 0.75 (0-2.75)	0.942
SJC[μ, β]	0.71±2.28 0 (0-21)	0.79±2.54 0 (0-21)	0.48±1.35 0 (0-6)	0.343
ESR (mm/h)[μ, β]	20.13±13.78 18 (2-90)	20.31±14.22 18 (2-90)	19.65±12.72 18 (4-46)	0.936
CRP (mg/dL)[μ, β]	11.33±15.16 5.82 (0.17-72.72)	11.98±15.89 6.5 (0.2-72)	9.54±13.05 4.54 (0.17-60.5)	0.458
van der Heidje erosion score[μ, β]	6.61±11.76 2.5 (0-71)	6.86±12.09 2.5 (0-71)	5.93±11.01 2.5 (0-57.50)	0.887
van der Heidje joint space narrowing score[μ, β]	15.96±12.17 13.5 (0-56)	16.93±12.57 14 (1-56)	13.34±10.77 13 (0-38.5)	0.182
DAS 28[μ, β]	3.19±1.37 3.01 (0.49-6.55)	3.20±1.37 3.01 (0.49-6.55)	3.15±1.39 3.25 (0.97-6.28)	0.862

Table 3: Comparison of clinical, laboratory, and radiological characteristics in respect to vitamin D status.

[μ]: mean±standard deviation, [π]: n (%), [β]: median (min-max)
SD: Standart deviation, VAS: visual analogue scale, TJC: tender joint count, RAQoL: RA Quality of Life Questionnaire, FSS: fatigue severity scale HAQ: Health Assessment Questionnaire, SJC: swollen joint count, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, DAS28: Disease Activity Score 28.

The ratios of severe deficient, deficient, and non-deficient vitamin D levels for RA patients were 40%, 33%, and 27%, respectively. The ratios of severe deficient, deficient, and non-deficient vitamin D levels for non-RA controls were 16%, 36%, and 48%, respectively. There were significant differences between groups in respect of vitamin D status (p=0.005).

Demographic and clinical, laboratory and radiological characteristics in RA individuals are summarized in Table 2 and Table 3, respectively, according to vitamin D status category. Age, gender, BMI, waist circumference, smoking status, disease duration, positivity of RF and anti-CCP were not different between groups. No significant difference was determined among the vitamin D status groups in patient reported subjective assessments (Duration of morning stiffness, VAS, number of tender joints, RAQoL, FSS, HAQ scores), and in objective assessments (SJC, ESR, CRP, radiologic damage scores) (Table 2-3). There was no correlation between DAS28-ESR and the levels of 25(OH) D (p=0.862). Additionally, we demonstrated that the serum concentrations of vitamin D were not significantly different when evaluated according to grades of disease activity (Figure 2).



DAS28: Disease Activity Score 28

Figure 2: Distribution of Vitamin D levels (ng/ml) (Y axis) according to the disease activity scores based on DAS 28.

Discussion

The importance of vitamin D in RA is a still mystery due to conflicting results in the literature. It is another debatable issue whether the impact of vitamin D on disease activity in RA occurs by patient perception effect rather than immune modulator effect. For this reason, the purpose of the current study was to analyze the impact of vitamin D in both the subjective and the objective components in established RA. Our study demonstrated that although vitamin D insufficiency was significantly higher in RA participants than control group, we did not observe any impact of vitamin D deficiency on objective assessments or even subjective assessments.

In countries near equator, where humans synthesize vitamin D on their skin for longer periods during the year, the low incidence of rheumatic disease prompted to research the role of vitamin D on the existence of rheumatic diseases [19]. Besides, an experimental study in 1998 showed that vitamin D receptor agonists reduced disease expression and worsening of arthritis [20]. Thus, various studies to date which had inconsistent results have been conducted to investigate whether there is a connection between the presence of rheumatic diseases and vitamin D level [1, 21]. Factors such as age, gender, BMI, smoking status, living environment, season, receiving vitamin D supplementation may affect the results. Therefore, in order to decrease the influence of confounding factors, we chose healthy controls from patients' relatives who shared the similar living

environment and measured the level of vitamin D in winter to decrease the seasonal effect. We also excluded the participants who received vitamin D supplementation in the past 12 months. Our age-gender matched participants were also similar in terms of smoking status and BMI. Nevertheless, the current study showed that the vitamin D levels were lower in RA participants when compared with healthy participants. Similarly, RA participants had a higher rate of vitamin D deficiency than healthy controls, 73%, 52% respectively. This rate in RA patients is parallel to the data reported to date that demonstrated vitamin D deficiency achieving more than 80% [2]. Our findings were consistent with the recent two meta-analyses regarding the lower levels of D vitamin in RA [4, 19]. In addition to this, another meta-analysis demonstrated that vitamin D intake lowered the chance of developing RA [22]. In the light of all these data, vitamin D deficiency may have a role in the etiopathogenesis of RA, but we should state that low vitamin D levels in our established RA individuals may be dependent on reduced outdoor activities and, thus reduced sun exposure.

Previous studies of the relation among disease activity and vitamin D levels in RA participants demonstrated conflicting findings [19]. Our study demonstrated that serum vitamin D concentrations were not connected with disease activity, its individual objective and subjective components, inflammatory markers, RF and anti-CCP seropositivity, and radiological damage scores. We also assessed serum vitamin D concentration according to disease activity status, due to describing negative relationship between DAS28 and vitamin D levels in only the participants with active RA in some studies [23]. In this manner, we also found no significant differences according to disease activity categories. Although there have been many reports to analyze the connection between the disease activity and vitamin D levels in the RA participants, there were limited studies that conducted in the context of the levels of vitamin D and radiological damage, which is an objective indicator of disease severity. A well-powered longitudinal study by Baker et al. [7], found that vitamin D deficiency (<20 ng/ml) were not related to disease activity, inflammatory markers, and radiographic progression. Another longitudinal study demonstrated that D vitamin levels were associated with disease activity, fatigue, and morning stiffness, but any effect of vitamin D levels on radiographic damage was not shown [17]. Polasik et al. [5], also found no significant association among the vitamin D levels and either disease activity and radiologic damage in the RA participants. Finally, the other two studies showed that while lower vitamin D levels were related with disease activity, not related with radiological structural damage in RA [1, 24]. Although the above-mentioned studies, including ours; two of which were longitudinal, showed different results for various objective or subjective assessments regarding to RA and vitamin D deficiency, none of them found any significant effect of vitamin D deficiency on radiological damage, which is an important objective indicator of disease severity.

Higgins et al. [8] evaluated DAS28-ESR with and without VAS and showed that the level of vitamin D was only correlated with VAS. They emphasized that vitamin D deficiency might cause increased disease activity by affecting pain perception negatively. Therefore, we also evaluated the patients with parameters based on their perception. However, we did not determine any correlation of vitamin D level with the assessments related to patient perception (i.e. VAS, TJC, fatigue, morning stiffness, quality of life, functional disability) as well as in objective assessments (i.e. SJC, inflammatory markers, radiologic damage scores).

There are some limitations in the present study. Firstly, the cross-sectional character of our study did not permit for assessment of the cause-effect relation among vitamin D levels and components of RA. Secondly, we didn't question the patients in terms of their amount/frequency of outdoor activities and sunlight exposure. Finally, this present study included participants with a variable RA duration. Therefore, prospective longitudinal studies in the individuals with similar disease duration and sunlight exposure time are needed to explain impact of vitamin D on RA.

In conclusion, our study demonstrated that serum vitamin D concentrations were lower in RA patients. Vitamin D deficiency may play a role in the etiopathogenesis of RA, but we primarily attribute this result to the reduced outdoor activities and reduced sun exposure in RA individuals than healthy controls. This study also refuted the role of vitamin D on objective or subjective parameters in the patients with RA.

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Evaluation of relationship between disease severity, mean platelet volume, and platelet distribution width in stable chronic obstructive pulmonary disease

Stabil kronik obstrüktif akciğer hastalığında ortalama trombosit hacmi ve trombosit dağılım genişliğinin hastalık şiddeti ile ilişkisinin değerlendirilmesi

Pelin Uysal [1]

Abstract

Aim: This study was conducted to evaluate the relationship between disease severity, mean platelet volume and platelet distribution width in stable chronic obstructive pulmonary disease.

Methods: The study included 120 consecutive patients with stable chronic obstructive pulmonary disease and 30 consecutive age-matched healthy subjects (control group). Patients were classified as A (mild), B (mild to moderate), C (moderate to severe) and D (severe) defined by the GOLD committee and grouped as A/B (n= 60) and C/D (n=60).

Results: Platelet levels were not different among the groups. Mean platelet volume was lower in all patients than control group (p=0.001). Level of platelet distribution width was higher in all patients than control group (p=0.018). Mean platelet volume in C/D groups were significantly lower than A/B group (p=0.011) and control group (p=0.001). Mean platelet volume in A/B group were also significantly lower than control group (p=0.001). Erythrocyte sedimentation rates were higher in A/B and C/D groups than control group (p=0.007 and p=0.001, respectively). C-reactive protein levels in control group were significantly lower than C/D group (p=0.001). No statistically significant correlations were observed between mean platelet volume and forced expiratory volume in one second and forced expiratory volume in one second/forced vital capacity or between mean platelet volume and other inflammatory parameters in A/B or C/D groups. Significant positive correlations were found between erythrocyte sedimentation rate and C-reactive protein (r=0.375; p=0.003), and between mean platelet volume and platelet large cell ratio (r=0.749; p=0.001) in C and D groups.

Conclusion: It was concluded that mean platelet volume could be used as a negative acute phase reactant in evaluation of disease severity of chronic obstructive pulmonary disease as C-reactive protein.

Keywords: Chronic obstructive pulmonary disease, mean platelet volume, platelet distribution width, forced expiratory volume, forced vital capacity.

Öz

Amaç: Bu çalışmada kronik obstrüktif akciğer hastalığında ortalama trombosit hacmi ve trombosit dağılım genişliği ile hastalık şiddeti arasındaki ilişkinin değerlendirilmesi amaçlandı.

Yöntemler: Çalışmaya 120 ardışık kronik obstrüktif akciğer hastası ve yaşları eşleştirilmiş 30 ardışık sağlıklı kişi (kontrol grubu) alındı. GOLD komitesi tarafından tanımlanan kronik obstrüktif akciğer hastalığı şiddetini belirlemek için hastalar A (hafif), B (hafif-orta), C (orta-şiddetli) ve D (şiddetli) olarak sınıflandı ve A/B (n=60) ve C/D (n=60) olarak gruplandı.

Bulgular: Gruplar arasında trombosit düzeylerinin istatistiksel olarak anlamlı olmadığı bulundu. Ortalama trombosit hacmi tüm hastalarda kontrollere göre daha düşüktü (p=0,001). Tüm hastalarda trombosit dağılım genişliği düzeyleri kontrol grubundan daha yüksekti (p=0,018). Ortalama trombosit hacmi C/D grubunda, A/B grubu (p=0,011) ve kontrol grubundan (p=0,001) anlamlı olarak düşük bulundu. Ortalama trombosit hacmi, A/B grubunda kontrol grubundan anlamlı derecede düşük bulundu (p=0,001). Eritrosit sedimantasyon hızı A/B ve C/D gruplarında kontrol grubundan daha yüksek iken (sırasıyla, p=0,007 ve p=0,001). C-reaktif protein düzeyleri kontrol grubunda C/D grubundan anlamlı derecede düşük bulundu (p=0,001). A/B veya C/D gruplarında ortalama trombosit hacmi ile bir saniyedeki zorlu ekspiratuar hacim, bir saniyedeki zorlu ekspiratuar hacim/zorlu vital kapasite oranı ve diğer inflamatuar parametreler arasında istatistiksel olarak anlamlı bir ilişki görülmedi. C/D grubunda eritrosit sedimantasyon hızı, C-reaktif protein düzeyleri (r=0.375; p=0,003) ile ve ortalama trombosit hacmi de büyük hücreli platelet oranı ile (r=0,749; p=0,001) pozitif korelasyon gösterdi.

Sonuç: Bu çalışma kronik obstrüktif akciğer hastalığında klasik pozitif akut faz reaktanı C-reaktif proteinin yanı sıra düşük ortalama trombosit hacmi'nin hastalık şiddetinin değerlendirilmesinde negatif akut faz reaktanı olarak kullanılabileceğini düşündürmektedir.

Anahtar sözcükler: Kronik obstrüktif akciğer hastalığı, ortalama trombosit volümü, trombosit dağılım genişliği, bir saniyedeki zorlu ekspiratuar hacim, zorlu vital kapasite.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the limitation of fully reversible airflow. COPD is a very common disease with high mortality. The development of COPD is contributed by genetic and environmental factors, together with progressive and systemic inflammation [1].

Currently, it is known that COPD patients also have systemic effects that are not only localized to the lungs [2, 3]. Patients with COPD and other chronic airway diseases may also have pulmonary hypertension, pulmonary embolism, and thrombocyte dysfunction at the same time; some COPD studies have indicated platelet function disorders and activation in the coagulation system [4, 5]. Mean platelet volume (MPV) is one of the markers of platelet activation that was reported to be associated with inflammation in recent studies [6, 7]. Intensive production of proinflammatory cytokines and acute phase reactants has been reported to affect MPV levels as a megakaryopoiesis inhibitor [8]. It has been reported to be high in some studies in COPD, but low in others [9].

MPV is known to be an early marker of platelet activation. Activated platelets are very important in inflammation, atherogenesis, and atherothrombosis. COPD is associated with cardiovascular disease-related mortality [10]. Platelets, together with their indices of MPV, platelet distribution width (PDW) can be used as markers of inflammation in cardiovascular, inflammatory, and thromboembolic diseases [11, 12]. PDW shows the standard deviation of the logarithmic transformation of platelets. In one study, PDW levels in COPD and pulmonary embolism were found to be elevated, but not associated with disease severity [13].

Despite this widespread and mortal illness, number of cheap and easily accessible parameters currently used in COPD is limited. In this study, we aimed to evaluate the relationship between disease severity and routine parameters such as MPV, PDW in whole blood tests in stable COPD patients.

Material and methods

The study was approved by the Ethics Committee (24.08.2017, 2017/14) and written informed consent was obtained from each subject. All participants were informed about the study and signed the consent form. This was a retrospective case-control study conducted in the Acibadem University School of Medicine, Acibadem Atakent Hospital, Department of Chest Diseases. A total of 120 consecutive COPD patients and 30 consecutive age-matched control subjects between June 2016 and October 2017 were enrolled in the study.

All the controls with normal pulmonary function tests were recruited from outpatient clinic of our hospital. All the subjects underwent physical examination. Subjects with chronic disease such as diabetes mellitus, hypertension, coronary artery disease, heart failure, irritable bowel disease were not included in the study.

The COPD patients were diagnosed based on the 2016 Global Initiative for Chronic Obstructive Lung Disease (GOLD), and they were classified into four groups (GOLD I/II/III/IV) according to the revised GOLD guidelines based on post-bronchodilator forced expiratory volume in one second (FEV1). According to this classification system (classification of severity of airflow), patients with FEV1 to forced vital capacity (FEV1/FVC) <0.70 were grouped as GOLD I (Mild, FEV1≥80% predicted), GOLD II (Moderate, 50% ≤ FEV1 < 80% predicted), GOLD III (Severe, 30% ≤ FEV1<50% predicted), and GOLD IV (Very severe, FEV1 < 30% predicted) [14]. Patients were divided into four groups [A (mild) / B (mild to moderate), n=60); C (moderate to severe) / D (severe), n=60)] according to the evaluation to determine COPD severity as defined by the GOLD committee. A combined assessment system of COPD severity was used for the classification of patients.

Exclusion criteria for all the patients were respiratory disorders other than COPD, pulmonary embolism, and left ventricular systolic or diastolic dysfunction, comorbidities such as cancer, diabetes, chronic renal insufficiency, hyperthyroidism, hypothyroidism, hepatic dysfunction, lower respiratory tract infection, or COPD attack in the last 6 week, and presence of metabolic syndrome. Detailed medical history was obtained from all participants, and physical examination was performed.

Spirometry tests were done in accordance with the criteria recommended by the European Respiratory Society using computer-assisted spirometry (Vmax22D, Sensor Medics, California, USA). Pulmonary function parameters FEV1, FVC, and FEV1/FVC ratio were measured, and the absolute values and the percentage of expected values of these parameters were analyzed.

Blood samples were obtained before drug use in the morning. Samples were collected in EDTA-containing tubes and anticoagulant-free tubes after an overnight fast. After immediate centrifugation at 3000 g for 10 minutes, at 4 °C, plasma and serum samples were separated in Eppendorf tubes and frozen immediately at -80 °C until analysis.

Complete blood count parameters [White blood cell (WBC) (× 103/μL), hemoglobin (Hb) (g/dL), hematocrit (Htc) (%), platelet (PLT) (×103/μL), MPV (fL), PDW (fL), platelet-large cell ratio (P-LCR) (%), plateletcrit (PCT) (%)] were obtained with automatic hematology analyzer (Siemens-Sysmex, Germany). Serum C-reactive protein (CRP) (mg/L) levels were measured by nephelometry (Siemens-Dimention, Germany). Erythrocyte sedimentation rate (ESR) (mm/h) was measured according to the Westergren method with an established normal range of 0–20 mm/h.

Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 20.0 package program was used for statistical evaluations. The relationship between the categorical variables of the groups was examined by chi-square test. Descriptive statistics were obtained, and data were tested for normality using the Kolmogorov-Smirnov test for Gaussian distribution. For comparison of parameters with normal distribution, parametric tests and comparison of parameters with abnormal distribution, non- parametric tests were used. For this purpose, One-Way ANOVA, unpaired student- t, Kruskal-Wallis and Mann-Whitney U tests were used. Relationships between variables were assessed with Pearson's or Spearman's correlation coefficient. The minimal significance (α) and statistical power (1 − β) were set at 0.05 and 0.80 respectively. A p value equal to or lower than 0.05 was considered as statistically significant.

Results

The baseline characteristics of the study groups are presented in Table 1. FEV1 (%) and FEV1/FVC were lower in patients than control group. WBC (×103/μL), ESR (mm/h) and CRP (mg/L) levels in patient groups were significantly higher than control group (p=0.001 for each). Hb (g/dL) and Htc (%) levels were lower in patient groups than control group (p=0.001 for each). MPV (fL) levels in patient groups were significantly lower than control group (p=0.001). PDW (fL) levels were higher in all patients than control group (p=0.018).

Clinical and laboratory findings in the patients according to the GOLD stage are given in Table 2. Both A/B

group and C/D group have lower FEV1 (%) and FEV1/FVC levels than control group (p=0.001 for each comparison). FEV1 (%) and FEV1/FVC were lower in C and D groups than A and B groups. WBC (× 103/μL) levels in control group and A/B group p=0.002 and p=0.026, respectively) were significantly lower than C/D group. Hb (g/dL) and Htc (%) levels were lower in patient groups than control group (p=0.001 for each). MPV (fL) levels were lower in all patients than control group (p=0.001). However, PDW (fL) levels were higher in all patients than control group (p=0.007). MPV (fL) levels in C/D group were significantly lower than A/B group (p=0.011) and control group (p=0.001). MPV (fL) levels in A/B group were also significantly lower than control group (p=0.001). Furthermore, PDW (fL) levels in control group were significantly lower than A/B and C/D groups (p=0.015 and p=0.007, respectively). While ESR (mm/h) levels were higher in A/B and C/D groups than control group (p=0.007 and p=0.001, respectively); in A/B group, they were lower than C/D group (p=0.049). CRP (mg/L) levels in control group were significantly lower than C/D group (p=0.001).

In all subjects, FEV1 (%) positively correlated with FEV1/FVC (r=0.850, p=0.001), Hb (g/dL) (r=0.396, p=0.001) Htc (%) (r=0.291, p=0.001), MPV (fL) (r=0.323, p=0.001) and p-LCR (%) (r=0.181, p=0.027); and very weakly negatively correlated with ESR (mm/h) (r=-0.297, p=0.000) and CRP (mg/L) (r=-0.270, p=0.001). FEV1/FEVC positively correlated with Hb (g/dL) (r=0.390, p=0.001), Htc (%) (r=0.272, p=0.001), MPV (fL) (r=0.371, p=0.001) and p-LCR (%) (r=0.213, p=0.009); also negatively correlated with ESR (mm/h) (r=-0.281, p=0.001) and CRP (mg/L) (r=-0.223, p=0.013). Also, WBC (× 103/μL) levels in all subjects were found as very weakly negatively correlated with FEV1 (%) (r=-0.246, p=0.002) and FEV1/FVC (r=-0.276, p=0.001). Correlation data for all subjects are given in Table 3.

Significant weak correlation was found between ESR (mm/h) and CRP (mg/L) (r=0.474, p=0.000) in A and B groups. Furthermore, in this group, ESR (mm/h) was very weakly negatively correlated with Hb (g/dL) (r=-0.294, p=0.023), Htc (%) (r=-0.311, p=0.016), and WBC (× 103/μL) levels (r=-0.355, p=0.005). MPV (fL) was also found as strongly positively correlated with p-LCR (%) (r=0.988, p=0.001), and very weakly positively correlated with WBC (× 103/μL) levels (r=0.302, p=0.019) (Table 4). While ESR (mm/h) level was negatively correlated with Hb (g/dL) (r=-0.276; p=0.033), it was positively correlated with CRP (mg/L) (r=0.375; p=0.003) in C and D groups. Also in this group, strongly positive correlations were found between MPV (fL) and p-LCR (%) (r=0.749, p=0.001); Hb (g/dL) and Htc (%) (r=0.916, p=0.000); and moderately positive correlation was present between FEV1 (%) and FEV1/FVC (r=0.628, p=0.001) (Table 5).

Table 1. Demographic, clinical and laboratory findings of the groups.

Variable	Control group (n=30)	All patients (n=120)	p
Age (year)	54.6±3.2	54.2±5.3	0.693
F/M	13/17	37/83	0.196
FEV1 (%)	102.0±8.9	56.7±24.2	0.001
FEV1/FVC	83.4±4.5	58.4±11.6	0.001
WBC (× 10^3/μL)	7.2±1.6	8.7±3.0	0.001
Hb (g/dL)	15.2±1.4	13.2±2.1	0.001
Htc (%)	45.7±4.2	41.0±5.6	0.001
PLT (×10^3/μL)	254.1±64.12	255.7±87.0	0.923
MPV (fL)	10.3±0.8	9.4±0.8	0.001
PDW(fL)	12.7±2.4	11.5±1.7	0.018
P-LCR (%)	25.6±6.1	22.7±5.8	0.021
PCT (%)	0.25±0.1	0.25±0.1	0.619
ESR (mm/h)	10.7±8.2	21.6±13.6	0.001
CRP (mg/L)	0.32±0.2	0.62±0.6	0.001

FEV1: Forced expiratory volume in the first second, FVC: Forced vital capacity, WBC: White blood cell, Hb: Hemoglobin, Htc: Hematocrit, PLT: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width, P-LCR: Platelet large cell ratio, PCT: Plateletcrit, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein.

Table 2. Clinical and laboratory findings of the groups according to the GOLD stage.

Variable	Control group (n=30)	A/B group (n=60)	C/D group (n=60)	p		
				Control vs A/B	Control vs C/D	A/B vs C/D
Age (year)	54.6±3.2	54.2 ±6.8	54.2 ±3.2	0.777	0.609	0.959
F/M	13/17	22/38	15/45	0.546	0.078	0.169
FEV1 (%)	102.0±8.9	77.5 ±13.9	36.0 ±10.3	0.001	0.001	0.001
FEV1/FVC	83.4±4.5	66.2 ±6.1	5765±10.3	0.001	0.001	0.001
WBC (×10^3/μL)	7.2±1.6	8.1 ±2.5	9.3±3.3	0.105	0.002	0.026
Hb (g/dL)	15.2±1.4	13.7 ±2.1	12.7 ±2.0	0.001	0.001	0.012
Htc (%)	45.7±4.2	41.6 ±5.2	40.3 ±6.0	0.001	0.001	0.215
PLT (×10^3/μL)	254.1±64.2	252.8 ±68.6	258.6±102.7	0.935	0.827	0.720
MPV (fL)	10.3±0.8	9.7 ±0.8	9.3±0.7	0.001	0.001	0.011
PDW(fL)	11.4 ±1.9	11.7 ±1.5	12.7±2.4	0.015	0.007	0.405
P-LCR (%)	25.6±6.1	23.3 ±5.3	22.1 ±6.3	0.066	0.012	0.247
PCT (%)	0.25±0.1	0.24 ±0.1	0.25±0.1	0.587	0.482	0.318
ESR (mm/h)	10.7±8.2	18.5 ±12.3	24.7±14.9	0.007	0.001	0.049
CRP (mg/L)	0.32±0.2	0.52 ±0.5	0.78 ±0.6	0.062	0.001	0.080

FEV1: Forced expiratory volume in the first second. FVC: Forced vital capacity. WBC: White blood cell. Hb: Hemoglobin. Htc: Hematocrit. PLT: Platelet. MPV: Mean platelet volume. PDW: Platelet distribution width. P-LCR: Platelet large cell ratio. PCT: Plateletcrit. ESR: Erythrocyte sedimentation rate. CRP: C-reactive protein.

Discussion

COPD is an important public health problem in our country as in other countries. Many studies have shown that there is an obvious increase in inflammation in airways during exacerbations among COPD patients who are generally categorized as mild, moderate and severe [14, 15]. Recently, MPV has been used as a simple and promising marker in several inflammatory circumstances. While MPV levels were lower in all patients than controls, PDW levels were higher in all patients than controls. MPV levels in C/D group were significantly lower than A/B group and control group. MPV levels in A/B group were also significantly lower than control group. The decreased MPV could be used as a negative acute phase reactant in the evaluation of disease severity in COPD as well as the classic positive acute phase reactant CRP.

Inflammatory parameters as WBC, ESR and CRP levels in patient groups were significantly higher than control group in the current study. WBC levels were significantly negatively correlated with ESR in A and B groups, and with FEV1 and FEV1/FVC in all subjects. ESR levels were positively correlated with CRP in C and D groups. Milacić et al. [16] showed that the higher the CRP level, the higher was the class of disease severity according to GOLD and the CRP value to correlate with the severity of the COPD clinical presentation. However, the leukocyte count did not show any significant correlation with the severity of COPD. No statistically significant correlations were found between the level of CRP, the leukocyte count, and comorbidities. Simonovska et al. [17] found statistically significant differences in the mean value of CRP in patients with different level of bronchial obstruction. For many years, an easily measurable and noninvasive parameter which might reflect systemic inflammation has been researched. CRP has been the most selective biomarker among the 36 plasma biomarkers for confirming COPD exacerbation and predicting COPD severity as our study [19]. WBC, ESR, and CRP are still the most frequently used infection markers in daily clinical practice [18].

The parameters related to the platelet size reflect platelet activity and are termed the platelet indices. The routinely available indices which describe platelet morphology and function are PLT, the platelet-to-lymphocyte ratio (PLR), MPV, and PDW [20]. But, in the current study, PLT of control subjects and patient groups revealed no significant difference, while MPV levels in patient groups were significantly lower than control group. PDW levels were higher in all patients than control group.

MPV levels in C/D group were significantly lower than A/B group and control group. MPV levels in A/B group were also

Table 3. Correlations for biochemical parameters in all subjects.

	FEV1 (%)	FEV1 / FVC	WBC ($\times10^3/\mu L$)	Hb (g/dL)	Htc (%)	MPV (fL)	P-LCR (%)	CRP (mg/L)
FEV1 (%)	-	r=0.850 p=0.001	r=-0.246 p=0.002	r=0.396 p=0.001	r=0.291 p=0.001	r=0.323 p=0.001	r=0.181 p=0.027	r= -0.270 p=0.001
FEV1/FVC	r=0.850 p=0.001	-	r=-0.276 p=0.001	r=0.390 p=0.001	r=0.272 p=0.001	r=0.371 p=0.001	r=0.213 p=0.009	r=-0.203 p=0.013
WBC ($\times10^3/\mu L$)	r=-0.246 p=0.002	r=-0.276 p=0.001	-	r=-0.044 p=0.591	r=0.002 p=0.977	r=-0.005 p=0.951	r=0.084 p=0.306	r=0.190 p=0.020
Hb (g/dL)	r=0.396 p=0.001	r=0.390 p=0.001	r=-0.044 p=0.591	-	r=0.925 p=0.001	r=0.203 p=0.013	r=0.110 p=0.179	r=-0.329 p=0.001
Htc (%)	r=0.291 p=0.001	r=0.272 p=0.001	r=0.002 p=0.977	r=0.925 p=0.001	-	r=0.165 p=0.043	r=0.150 p=0.067	r=-0.235 p=0.004
MPV (fL)	r=0.323 p=0.001	r=0.371 p=0.001	r=-0.005 p=0.951	r=0.203 p=0.013	r=0.165 p=0.043	-	r=0.800 p=0.001	r=-0.308 p=0.001
P-LCR (%)	r=0.181 p=0.027	r=0.213 p=0.009	r=0.084 p=0.306	r=0.110 p=0.179	r=0.150 p=0.067	r=0.800 p=0.001	-	r=-0.172 p=0.035
ESR (mm/ h)	r=-0.297 p=0.001	r=-0.281 p=0.001	r=-0.012 p=0.885	r=-0.383 p=0.001	r=-0.345 p=0.001	r=-0.226 p=0.005	r=-0.120 p=0.144	r=0.446 p=0.001

FEV1: Forced expiratory volume in the first second. FVC: Forced vital capacity. WBC: White blood cell. Hb: Hemoglobin. Htc: Hematocrit. PLT: Platelet. MPV: Mean platelet volume. P-LCR: Platelet large cell ratio. ESR: Erythrocyte sedimentation rate. CRP: C-reactive protein.

Table 4. Correlations for biochemical parameters in A and B groups.

	FEV1 (%)	FEV1 / FVC	WBC ($\times10^3/\mu L$)	Hb (g/dL)	Htc (%)	MPV (fL)	P-LCR (%)	CRP (mg/L)
FEV1 (%)	-	r=0.312 p=0.015	r=0.149 p=0.257	r=0.125 p=0.342	r=0.142 p=0.281	r=0.030 p=0.822	r=0.082 p=0.535	r=0.046 p=0.724
FEV1 / FVC	r=0.312 p=0.015	-	r=0.112 p=0.394	r=0.218 p=0.095	r=0.168 p=0.200	r=0.135 p=0.302	r=0.131 p=0.320	r=0.155 p=0.236
WBC ($\times10^3/\mu L$)	r=0.149 p=0.257	r=0.112 p=0.394	-	r=0.040 p=0.762	r=0.086 p=0.516	r=0.302 p=0.019	r=0.309 p=0.016	r=0.084 p=0.523
Hb (g/dL)	r=0.125 p=0.342	r=0.218 p=0.095	r=0.040 p=0.762	-	r=0.952 p=0.001	r=-0.032 p=0.807	r=-0.040 p=0.763	r=-0.220 p=0.091
Htc (%)	r=0.142 p=0.281	r=0.168 p=0.200	r=0.086 p=0.516	r=0.952 p=0.001	-	r=-0.044 p=0.741	r=-0.042 p=0.748	r=-0.175 p=0.182
MPV (fL)	r=0.030 p=0.822	r=0.135 p=0.302	r=0.302 p=0.019	r=-0.032 p=0.807	r=-0.044 p=0.741	-	r=0.988 p=0.001	r=-0.184 p=0.159
P-LCR (%)	r=0.082 p=0.535	r=0.131 p=0.320	r=0.309 p=0.016	r=-0.040 p=0.763	r=-0.042 p=0.748	r=0.988 p=0.001	-	r=-0.166 p=0.206
ESR (mm/h)	r=-0.038 p=0.776	r=0.034 p=0.796	r=-0.355 p=0.005	r=-0.294 p=0.023	r=-0.311 p=0.016	r=-0.146 p=0.265	r=-0.142 p=0.281	r=0.474 p=0.001

FEV1: Forced expiratory volume in the first second. FVC: Forced vital capacity. WBC: White blood cell. Hb: Hemoglobin. Htc: Hematocrit. PLT: Platelet. MPV: Mean platelet volume. P-LCR: Platelet large cell ratio. ESR: Erythrocyte sedimentation rate. CRP: C-reactive protein.

Table 5. Correlations for biochemical parameters in C and D groups.

	FEV1 (%)	FEV1 /FVC	WBC ($\times10^3/\mu L$)	Hb (g/dL)	Htc (%)	MPV (fL)	P-LCR (%)	CRP (mg/L)
FEV1 (%)	-	r=0.628 p=0.001	r=-0.180 p=0.169	r=-0.008 p=0.954	r=-0.099 p=0.451	r=-0.114 p=0.386	r=-0.055 p=0.675	r=0.225 p=0.084
FEV1 /FVC	r=0.628 p=0.001	-	r=-0.222 p=0.089	r=-0.130 p=0.324	r=-0.189 p=0.147	r=0.095 p=0.471	r=0.050 p=0.703	r=0.164 p=0.211
WBC ($\times10^3/\mu L$)	r=-0.180 p=0.169	r=-0.222 p=0.089	-	r=0.136 p=0.300	r=0.134 p=0.309	r=-0.018 p=0.889	r=-0.010 p=0.942	r=0.209 p=0.109
Hb (g/dL)	r=-0.008 p=0.954	r=-0.130 p=0.324	r=0.136 p=0.300	-	r=0.916 p=0.001	r=0.051 p=0.699	r=0.063 p=0.635	r=-0.288 p=0.026
Htc (%)	r=-0.099 p=0.451	r=-0.189 p=0.147	r=0.134 p=0.309	r=0.916 p=0.001	-	r=0.015 p=0.911	r=0.152 p=0.248	r=0.184 p=0.159
MPV (fL)	r=-0.114 p=0.386	r=0.095 p=0.471	r=-0.018 p=0.889	r=0.051 p=0.699	r=0.015 p=0.911	-	r=0.749 p=0.001	r=-0.253 p=0.051
P-LCR (%)	r=-0.055 p=0.675	r=0.050 p=0.703	r=-0.010 p=0.942	r=0.063 p=0.635	r=0.152 p=0.248	r=0.749 p=0.001	-	r=-0.064 p=0.629
ESR (mm/h)	r=0.145 p=0.269	r=0.041 p=0.753	r=0.070 p=0.596	r=-0.276 p=0.033	r=-0.215 p=0.099	r=0.035 p=0.789	r=0.134 p=0.308	r=0.375 p=0.003

FEV1: Forced expiratory volume in the first second. FVC: Forced vital capacity. WBC: White blood cell. Hb: Hemoglobin. Htc: Hematocrit. PLT: Platelet. MPV: Mean platelet volume. P-LCR: Platelet large cell ratio. ESR: Erythrocyte sedimentation rate. CRP: C-reactive protein.

significantly lower than control group. MPV has been studied as an inflammatory marker in various diseases [21-23]. Previous studies have reported controversial results regarding evaluation of MPV in COPD [24-33]. The conflicting data might be due to the failure to rule out confounding factors such as body mass index, smoking status, and the use of medications such as statins and angiotensin-converting enzyme inhibitors [34]. In our study, we ruled out respiratory disorders other than COPD, pulmonary

embolism, and left ventricular systolic or diastolic dysfunction, comorbidities such as diabetes, chronic renal insufficiency, hyperthyroidism, hypothyroidism, hepatic dysfunction and various drugs. Kalemci et al. [28] found a significant increase in PDW, MPV, platelets, PLR, and RDW, while mean platelet volume ratio decreased as the COPD severity increases. According to their results, patients with more severe COPD had higher MPV values, unlike our study. Wang et al. [9] found that COPD patients during exacerbation and in stable phase had lower MPV compared to healthy controls. MPV of participants with COPD was higher once patients had recovered from exacerbations. Moreover, reduced MPV was positively related to WBC and CRP levels in exacerbated COPD, not in the stable phase. Ulaslı et al. [26] reported that MPV values were significantly lower in patients during acute exacerbation than in those during the stable period of COPD and in control subjects. They announced that assessment of MPV in COPD exacerbation might indicate systemic inflammation and MPV might be used as a negative acute phase reactant in COPD exacerbation as in our results. There was no correlation between MPV and other inflammatory parameters, FEV1 and FEV1/FVC in stable phase in the current study. COPD may be associated with reduced MPV, independent of clinical manifestations.

PDW is the standard deviation of the logarithmic transformation of platelets. It is an index that provides information about the viability of the platelets to be used in transfusions [28]. Recent studies have reported that there was an increase in PDW, MPV, and PCT values with an increase in the severity of COPD (from A to D) [28]; however, the results were contradictory. Wang et al. [12] reported that a significant increase in PDW was related to COPD with pulmonary embolisms. However, they did not observe a relationship between PDW and disease severity as in our study. PDW levels in control group were significantly lower than A/B and C/D groups in our study. We determined that PDW was independently associated with severe COPD and clinical parameters. By contrast, Steiropoulos et al. [29] noticed no significant differences in MPV and PDW values between patients with different stages of COPD. They found that a significant correlation was noted between MPV and WBC in patients with COPD, and especially in those with severe COPD stage III and very severe COPD stage IV. Analysis of Białas et al. [19] indicated that elevated PDW was associated with reduced survival of patients with COPD and PDW might be used as an inexpensive and repeatable prognostic tool in COPD. Makhlouf et al. [25] reported that PDW, PCT, and CRP were significantly higher in COPD patients, either nondiabetic or diabetic. Koc et al. [35] found that MPV and PDW levels were lower, and WBC was higher in patients with COPD compared to smokers and nonsmoking subjects; however, the platelet count was not significantly different.

This study also has some limitations. First, our sample size is relatively small. Second, dietary habits, physical activity and the exercise level of the subjects were not documented. Third, correlations of systemic blood count parameters with pulmonary function test were relatively weak.

Although the association between MPV, PDW, and COPD was controversial, our results suggested that MPV was decreased and PDW was elevated in patients with COPD. MPV could partly reflect disease severity, not PDW. Platelets play an important role in inflammatory conditions related to COPD. Chronic inflammation is also known to induce platelet activation. Platelet function may be modified by the systemic inflammation associated with COPD. Low MPV count can be used as a negative acute phase reactant in the evaluation of disease severity in COPD as well as the classic positive acute phase reactant, CRP. However, we are of the opinion that the platelet count cannot be used for the evaluation of disease severity in COPD.

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VCAM1 (T-1591C and T-833C) and E-selectin S128R polymorphisms are not risk factors for Hashimoto's thyroiditis.

VCAM1 (T-1591C ve T-833C) ve E-selektin S128R polimorfizmleri Hashimoto tiroiditi için risk faktörleri değildir.

Sevgin Değirmencioğlu [1], Pervin Vural [2], Ayşenur Özderya [3], Semra Doğru Abbasoğlu [2]

Abstract

Aim: The etiopathogenesis of Hashimoto's thyroiditis (HT) has not been clearly elucidated although the role of chronical inflammation and endothelial dysfunction has been established. Adhesion molecules such as vascular cell adhesion molecule1 (VCAM1) and E-selectin are secreted from vascular endothelium and promote accummulation of leukocytes in damaged endothelial areas. This study examined the possible association of VCAM1 (T-1591C and T-833C) and E-selectin S128R single nucleotide polymorphisms (SNPs) with the occurence of HT for the first time.

Methods: VCAM1 (T-1591C and T-833C), and E-selectin S128R SNPs in DNA from peripheral blood leukocytes of 189 patients with HT and 247 healthy controls were investigated by real-time PCR combined with melting curve analysis using fluorescence-labeled hybridization probes.

Results: We did not find significant differences in the distributions of studied polymorphisms between patients with HT and healthy controls.

Conclusions: The results of present study suggest that VCAM1 (T-1591C and T-833C) and E-selectin S128R SNPs may not be risk factors for HT. For all that; further studies with a larger cohort, analyzing other polymorphisms in VCAM1 and E-selectin genes are necessary to support our observations.

Key words: Hashimoto's thyroiditis, VCAM1, E-selectin, polymorphism

Öz

Amaç: Hashimoto tiroiditinin (HT) etyopatogenezi tam olarak aydınlatılamamış olmakla birlikte kronik inflamasyon ve endotel disfonksiyonunun önemli olduğu bilinmektedir. Vasküler hücre adezyon molekülü 1 (VCAM1) ve E-selektin gibi adezyon molekülleri endotel tarafından salgılanırlar ve hasarlı endotel bölgesine lökositlerin migrasyonunu düzenlemektedirler. Bu çalışmada ilk kez VCAM1 (T-1591C ve T-833C) ve E-selektin S128R tek nükleotid polimorfizmleri (SNPs) ile HT arasında bir ilişki olup olmadığı araştırılmıştır.

Metod: HT'li 189 hasta ve 247 sağlıklı kontrol kişinin periferik kan lökositlerinden izole edilen DNA örneklerinde VCAM1 (T-1591C ve T-833C) ile E-selektin S128R polimorfizmleri floresan boya-işaretli problar kullanan ve erime eğri analizine dayanan "real-time" PCR yöntemi ile incelendi.

Bulgular: Çalışmamızda kontrol grubu ve HT hastalarında araştırılan polimorfizmlerin dağılımlarında anlamlı bir fark bulunmadığı saptandı.

Sonuç: Bu çalışmanın bulgularına göre VCAM1 (T-1591C ve T-833C) ve E-selektin S128R polimorfizmleri HT için bir risk faktörü olmayabileceğini düşündürmektedir. Bununla birlikte, bulgularımızın desteklenmesi için örnek sayısının arttırılması, ayrıca VCAM1 ve E-selektin genlerin farklı polimorfik lokuslarının da incelenmesi gerektiği kanısındayız.

Anahtar kelimeler: Hashimoto tiroiditi, VCAM1, E-selektin, polimorfizm

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Introduction

Hashimoto's thyroiditis is the most common organ-specific autoimmune disorder characterized by diffuse lymphocytic infiltration of the thyroid gland, elevated levels of the serum anti-thyroid antibodies, evidence of goitrous or atrophic gland, and frequent thyroid dysfunction in varying degrees [1]. The precise pathophysiologic mechanism of HT remains still unclear. The most relative evidence suggests that ongoing immune intolerance and low-grade chronic inflammation with subsequent endothelial damage are included in the pathogenesis [2, 3]. The damage of the vascular endothelium plays a significant role in the extravasation of blood leukocytes infiltrating of thyroid gland, thus leading to an autoimmune response. [4-6]. Vascular cell adhesion molecule 1 (VCAM1) and E-selectin are significant biomarkers of endothelial dysfunction, mediating the recruitment and transendothelial transmigration of inflammatory cells from the circulation during autoimmune and atherosclerotic process. Patients with in autoimmune thyroid disorders, increased expression and serum levels of adhesion molecules are found [7-9]. VCAM1(T-1591C and T-833C) and E-selectin S128R single nucleotide polymorphisms (SNPs) are among the main polymorphisms of VCAM1 and E-selectin genes, implicated as risk factors in some autoimmune conditions [11-12]. To our knowledge, there is no any literature regarding for the association between above mentioned loci and susceptibility to HT. Therefore, in the present study we aimed to investigate whether VCAM1 (T-1591C and T-833C) and E-selectin S128R SNPs could affect tendency to HT.

Material and methods

One hundred and eighty nine patients with the specification of HT and 247 healthy participants as control were inclusive in this prospective case-control study. The study was confirmed by the Institutional Review Board at Şişli Etfal Research and Training Hospital. The study complied with the principles of the Declaration of Helsinki. Informed consent was obtained from each subject.

Symptoms or findings in hypothyroiditis including mild stiffness of the enlarged thyroidal gland, increased or normal thyroid stimulating hormone (TSH) value, decreased or normal free tri-iodo-thyronine (FT3) or free thyroxine (FT4) values, and high levels of autoantibodies (anti-thyroid peroxidase antibody (anti-TPO), anti-thyroglobulin antibody (anti-Tg)) in screening tests were diagnosed as HT. Damage of echogenity in parenchyma and fibrotic separations and pseudo nodular view in Doppler ultrasonography of the thyroid gland were also found. Fine needle aspiration was only performed to score the patients having a thyroid nodule ≥1cm in diameter (n=17). All HT patients were subjected to thyroxine therapy until euthyroid state has been achieved. The thyroxine dose was arranged according to TSH concentrations checked regularly in three month gaps. The control group occurred of 247 individuals matched for age and sex. None of the controls had personal or family history of thyroid disease and goiter on examination; they had normal thyroid functions and were negative for thyroid autoantibodies. Exclusion criteria were the existence of any co-morbid cardiac, autoimmune, infectious, musculoskeletal or malignant disease and a recent history of operation or trauma.

Blood samples were taken in the morning subsequent to an overnight (12 h) fast. Peripheral venous blood samples were collected in plain tubes for routine biochemical analysis, and in EDTA-K3 for genotype analysis. Serum cholesterol, triglyceride, very low density lipoprotein (VLDL)-, low density lipoprotein (LDL)- and high density lipoprotein (HDL)-cholesterol measurements were performed on ISE 1800 DPP Roche autoanalyzer (Roche Diagnostics, Germany). Serum TSH, freeT3, free T4, anti-Tg and anti-TPO were measured on Modular EEE Electrod Elecsys Roche autoanalyzer (Roche Diagnostics, Germany). Serum soluble VCAM1 (sVCAM1) and serum soluble E-selectin (sE-selectin) levels were measured by commercially available ELISA kits (Diaclone, Besançon, France) according to the manufacturer's instructions. The normal reference ranges for laboratory parameters were: anti-TPO < 5.61 IU/mL, anti-TG < 4.11 IU/mL, TSH 0.35-4.94 mI/mL, free T3 2.63-5.7 pmol/L, free T4 9.01-18.02 pmol/L, cholesterol 130-200 mg/dL, triglyceride <150 mg/dL, HDL-C > 40 mg/dL, LDL-C 100-130 mg/dL. Body mass index (BMI) was calculated as the ratio between body weight and height (kg/m2).

Genomic DNA was isolated from peripheral blood leukocytes by using High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche Diagnostics, Germany). Detection of polymorphisms was made by rapid capillary PCR with melting curve analysis using fluorescence-labeled hybridization probes in a LightCycler (Roche Diagnostics, Germany). PCR primers and probes for melting point analyses are given in Table 2. Primers and probes were designed by Tib MolBiol (Berlin, Germany). Analysis was done in 20 □l volumes using glass capillaries. The PCR mix contained 2 µl of the genomic DNA, 2 µl of LCTM FastStart DNA Master HybProbe kit (Roche Diagnostics), 0.5-1 µM of each primer, 0.2-0.4 µM of each probe and 1.5-2.5 mM total MgCl2. Reaction conditions were as follows: initial denaturation at 95 °C for 10 min, then 45 cycles of denaturation at 95 °C for 1 s, annealing (due to primers), and elongation at 72 °C for 12 s. Melting curve analysis was done with an initial denaturating step at 95 °C for 5 s and 20 s at 40-45 °C, slow heating to 70-80°C, with a ramping rate of 0.1-0.15 °C/s and continuous fluorescence detection. Melting curves were evaluated by two independent observers who were blinded to the analysis of the clinical data. In addition, 10% of randomly selected samples were repeated independently to verify genotyping results and 100% concordance was found. SNP genotypes were tested for departures from HWE for controls and patients, and all polymorphisms were in HWE.

Statistical analysis

Statistical analyses were performed with SPSS 15.0 for Windows (Chicago, IL, USA). In addition, the NCSS 2000 statistical package (Kaysville, Utah, USA) was used to evaluate the power analysis. We had a 97% power to detect an effect size (W) of 0.20 using a 2 degrees of freedom (α= 0.05). Differences in genotype distributions and allele frequencies in the patients and the controls were compared for statistical significance using the chi-square (χ^2) test. The statistical significance for deviations from Hardy-Weinberg Equilibrium (HWE) was determined using the Pearson χ^2-test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (CIs). The wild-type genotype/allele served as a reference category. Mann-Whitney U, Kruskal-Wallis and Spearman correlation tests were used for the evaluation of clinical and biochemical parameters. The differences were considered significant if the value of probability (p) did not exceed 0.05.

Results

A total of 436 subjects were included in this case-control study. The mean age of HT patients was 40.99±12.96 years (range 16-78 years) (21 male, 168 female), and of controls was 37.9±9.96 years (range 18-67 years) (32 males, 215

females). Clinical characteristics and thyroid hormonal status of controls and HT patients are shown in Table 1. TSH, cholesterol, LDL-C, sVCAM levels and BMI were significantly increased in HT patients according to controls (p= 0.001 for all).

The genotypic and allelic distributions of the polymorphisms in the studied genes for patients and the controls are shown in Table 3. In this study, no statistically significant differences were found in genotype or allele distributions of all evaluated polymorphisms between the patients with HT and the controls (Table 3). With respect to plasma levels of adhesion molecules, sVCAM1 concentrations were higher and sE-selectin levels were unchanged in patients with HT according to controls (Table 1). When sVCAM and sE-selectin concentrations in HT patients were evaluated according to studied polymorphisms, no significant differences between genotypes were found (Table 4-6).

Table 1: Characteristics of controls and patients with Hashimoto's thyroiditis (HT).

	Control (n=247)	HT (n=189)	p
Age (years) ¥	37.9±9.96 (18-67)	40.99±12.96 (16-78)	0.705
HT onset$^{\mu}$			
< 40 year	-	98 (51.9)	-
>40 year	-	90 (47.6)	-
Gender$^{\mu}$			
Male	32 (13.0)	21 (11.1)	0.719
Female	215 (87.0)	168 (88.9)	0.488
Familial history$^{\mu}$	-	99 (52.4)	-
Smoking$^{\mu}$	107 (43.3)	97 (51.3)	0.070
BMI (kg/m^2)$^{\beta}$	24.7±4.89	27.55±5.70	0.001
Systolic BP (mmHg)$^{\beta}$	118.2±10.4	115.2±11.9	0.209
Diastolic BP (mmHg)$^{\beta}$	73.5±7.9	71.8±10.3	0.215
Anti-TPO (IU/mL)$^{\beta}$	-	625.4±370.4	-
Anti-Tg (IU/mL)$^{\beta}$	-	560.4±380.0	-
TSH (mIU/L)$^{\beta}$	1.66±0.8	4.0±2.7	0.001
FreeT_3 (pmol/L)$^{\beta}$	3.2±0.3	3.3±0.4	0.315
FreeT_4 (pmol/L)$^{\beta}$	13.2±2.7	13.5±3.9	0.235
Cholesterol (mg/dL)$^{\beta}$	177.24±36.80	192.71±35.00	0.001
Triglyceride (mg/dL)$^{\beta}$	102.86±55.89	113.18±52.98	0.113
HDL-C (mg/dL)$^{\beta}$	56.38±12.56	59.25±12.20	0.116
LDL-C (mg/dL)$^{\beta}$	100.35±32.09	115.81±30.12	0.001
sVCAM1 (ng/mL)$^{\beta}$	1,477.7±491.9	2,733.5±880.6	0.001
sE-selectin (ng/mL)$^{\beta}$	69.11±35.49	66.88±31.81	0.250

¥: mean±SD (range), μ: n (%), β: mean±SD.
BP: blood pressure, BMI: body mass index, HT: Hashimoto's thyroiditis, HDL-C: high density lipoproteine-cholesterol, LDL-C: low density lipoproteine-cholesterol, sVCAM1: soluble vascular cell adhesion molecule 1, TSH: thyroid-stimulating hormone

Table 2: Sequences of primers or probes employed in this study

VCAM1 T -1591C (rs1041163)
Primer 1 5'-TGATGATGACACAAACACTGT-3'
Primer 2 5'-GAAAAATAAGTTGGAGATGCT-3'
Probe 1 5'-GGGATCAGAAAAATTGATTCAGG-FL
Probe 2 5'-LC640-CTAGCTTATAAACAAGTAACCCAGAGGTCCT-3'
VCAM1 T-833C (rs3170794)
Primer 1 5'-CAGATGGATTCCATACACTTTCATT-3'
Primer 2 5'-GGACTGTAACTGAAATTGCTGC-3'
Probe 1 5'-AAGTTACCAATAATTTGGTTAAATTGCTGGA-FL
Probe 2 5'-LC640-TTGGAATTTTTTTGCATACTTAAATG-3'
E-Selectin S128R (rs5361)
Primer 1 5'-TGCTGATGTCTCTGTTGC-3'
Primer 2 5'-GGTCTCTACACATTCACCG-3'
Probe 1 5'-GCTTTGTATTTTCCGTAGCTGCCTGTACC-FL
Probe 2 5'- LC640-ATACATCCTGCCGTGGCC-3'

HT: Hashimoto's thyroiditis, VCAM1: vascular cell adhesion molecule1

Table 3. Distribution of genotypes and allele frequencies for Hashimoto's thyroiditis (HT) and control group.

	Controls n (%)	HT n (%)	OR (95 % CI)	p
VCAM1 T-1591C				
TT$^{\mu}$	169 (68.4)	121 (64.0)	1.0*	-
CT$^{\mu}$	68 (27.5)	61 (32.3)	1.25 (0.83-1.90)	0.288
CC$^{\mu}$	10 (4.1)	9 (3.7)	0.98 (0.36-2.64)	0.964
CT+CC	78	70	1.22 (0.82-1.82)	0.334
T allele frequency	0.82	0.80	1.0*	-
C allele frequency	0.18	0.20	1.14 (0.81-1.61)	0.446
VCAM1 T-833C				
TT$^{\mu}$	234 (94.7)	179 (94.7)	1.0*	-
CT$^{\mu}$	13 (5.3)	10 (5.3)	1.01 (0.43-2.35)	0.989
CC$^{\mu}$	0 (0)	0 (0)	1.31 (0.03-66.16)	1.00
CT+CC	13	10	1.01 (0.43-2.35)	0.989
T allele frequency	0.97	0.97	1.0*	-
C allele frequency	0.03	0.03	1.00 (0.44-2.32)	0.989
E-selectin S128R				
SS$^{\mu}$	205 (83.0)	147 (77.8)	1.0*	-
SR$^{\mu}$	40 (16.2)	40 (21.2)	1.40 (0.86-2.27)	0.179
RR$^{\mu}$	2 (0.08)	0 (0)	1.40 (0.19-10.01)	0.739
SR+RR	42	40	1.40 (0.87-2.25)	0.170
S allele frequency	0.91	0.89	1.0*	-
R allele frequency	0.09	0.11	1.35 (0.87-2.09)	0.184

*: Reference values for OR, $^{\mu}$: n (%), CI: Confidence interval, OR: Odds ratio, HT: Hashimoto's thyroiditis, VCAM1: Vascular cell adhesion molecule 1

Table 4: sVCAM1 (vascular cell adhesion molecule 1) and sE-selectin levels in patients with Hashimoto's thyroiditis in accordance with their genotypes of the VCAM1 T-1591C gene polymorphism.

	TT	CT + CC	p
sVCAM (ng/mL)$^{\beta}$	2,780.7 ± 891.1	2,673.3 ± 871.0	0.549
sE-selectin (ng/mL)$^{\beta}$	69.84 ± 32.32	60.24 ± 30.74	0.149

$^{\beta}$: mean±SD

Table 5: sVCAM1 (vascular cell adhesion molecule 1) and sE-selectin levels in patients with Hashimoto's thyroiditis in accordance with their genotypes of the VCAM1 T-833C gene polymorphism.

	TT	CT	p
sVCAM (ng/mL)$^{\beta}$	2,747.4 ± 862.1	2,373.7 ± 801.0	0.286
sE-selectin (ng/mL)$^{\beta}$	66.15 ± 32.27	78.99 ± 22.50	0.250

$^{\beta}$: mean±SD

Table 6: sVCAM1 (vascular cell adhesion molecule 1) and sE-selectin levels in patients with Hashimoto's thyroiditis in accordance with their genotypes of the E-selectin S128R gene polymorphism.

	SS	SR + RR	p
sVCAM (ng/mL)$^{\beta}$	2,584.8 ±788.1	3,007.8 ± 997.2	0.065
sE-selectin (ng/mL)$^{\beta}$	68.87 ± 31.68	61.89 ± 32.43	0.387

$^{\beta}$: mean±SD

Discussion

The present study was conducted to investigate whether VCAM1 (T-1591C and T-833C) and E-selectin S128R SNPs could affect tendency to HT. The variant allele frequencies of the studied polymorphisms in our control group were consistent with previous studies [12-14].

Low-grade chronic inflammation and endothelial damage with subsequent endothelial dysfunction, have been increasingly recognized as having a central role in hypothyroidism [2, 3]. Cellular adhesion molecules are well accepted as markers of low-grade inflammation and endothelial dysfunction [15]. Some clinical studies as well as our results indicate that plasma levels of VCAM1 are elevated in patients with HT [7, 8]. Additionally, enhanced expression of VCAM1 has been observed in immunocytological analyses of tissue from thyroid glands in patients with HT [9]. VCAM1 is expressed on the surface of several cell types, including leukocytes, endothelial cells, macrophages, myoblasts, and dendritic cells [15]. VCAM1, facilitating the adhesion of leukocytes and monocytes to and their transmigration through the activated endothelium, plays an important role in the early stages of vascular disease in conditions with chronic inflammation [15]. It has been suggested that increased expression of VCAM1 on activated endothelium may facilitate transmigration of monocytes and T lymphocytes across the endothelium [16] with subsequent release of inflammatory cytokines by endothelial cells and monocytes [17]. In turn, cytokines induce VCAM1 expression, which binds adjacent endothelial cells (via its ligand very late antigen-4), therefore potentiating autoimmune process [17]. VCAM1 -1591 and -833 SNPs are among the main polymorphisms in the promoter region of VCAM1 gene described in the literature, regarding for association with various diseases [18-20]. Idelman et al. [20] demonstrated that -1591 and -833 loci of VCAM1 gene are biologically active and could influence the progression of some diseases. However, in our study, there is no any significant difference in the allele and genotype frequencies of studied VCAM1 polymorphisms between HT and controls. Neither -1591 nor -833 genotypes didn't influence the plasma VCAM1 concentrations.

E-selectin is expressed on endothelial cells after activation by pro-inflammatory cytokines [21]. The most studied polymorphism of E-selectin gene is S128R (or A561C) SNP found to be an important risk factor for development of endothelial dysfunction [13, 22, 23]. The substitution of serine (S) to arginine (R) has been shown to decrease importantly binding specificity and capacity to carbohydrate molecules on leukocyte surface, leading to an increase in cellular adhesion two- to three-fold in comparison with wild-type gene [24]. The 128R allele may thus increase leukocyte adherence to endothelium contributing to the progression of endothelial dysfunction. Additionally, Mlekush et al. [25] demonstrated that E-selectin plasma levels in 128R allele carrying subjects were significantly higher than S128S homozygous ones. It has been previously shown that 128R allele is associated with an increased risk for systemic lupus erythematosus in Spanish and English populations [12]. In addition, Chen et al. has been demonstrated that some common variants of E-selectin gene (not including S128R polymorphism) are related with increased risk for Graves' disease in Chinese population [11]. It is seen from the results that there is not any association between S128R polymorphism of E-selectin gene and susceptibility to HT in our population, and that S128R polymorphism does not have impact serum E-selectin levels.

Interpretation of our findings, as in the case of all genetic studies, meets with certain limitations. The biological effects of studied polymorphisms remain to be elucidated. Further studies investigating the mechanisms of genetic control of VCAM -1591 / – 833 and E-selectin 128 expressions are needed to clarify the possible relationship between VCAM /E-selectin genes and autoimmune disorders.

As a conclusion, VCAM1 (-1591 and -833) and E-selectin 128 polymorphisms seem to be not related with HT, although this cannot be definitively excluded without further analysis in a larger study group combined with analysis of further polymorphisms in the VCAM1 and E-selectin genes.

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Is there a correlation between the biceps brachii muscle stiffness measured by elastography and severity of lymphedema in patients with breast cancer-related lymphedema?

Meme kanseri ilişkili lenfödemde lenfödem şiddeti ve elastografi ile ölçülen biseps kası sertliği arasında bir korelasyon var mı?

Hülya Aslan [1], Pınar Doruk Analan [2], Emine Kaya [2]

Abstract

Aim: Breast-cancer-related lymphedema (BCRL) causes symptoms such as swelling, heaviness, tightness, firmness, pain, numbness, or impaired mobility in affected arm and hand. It also predisposes patients to fibrosis, cellulitis, infections, lymphadenitis, and septicemia. Aim of this study was to analyze correlation between the biceps brachii muscle stiffness measured by shear wave elastography (SWE) and severity of the lymphedema.

Methods: This prospective study included 20 consecutive patients (mean age, 54.6±5.4 years) with having BCRL in the upper limb. Stiffness of the biceps brachii muscle was assessed by SWE. Shear wave speeds (SWS) of the biceps muscle on the affected side for each patient were measured. Severity of the lymphedema was determined by difference between diameters and volumes of affected and unaffected extremities. Correlations between the biceps brachii muscle stiffness measured by SWE and difference between diameters and volumes of affected and unaffected extremities were analyzed.

Results: SWS of the biceps muscle on the affected side showed positive fair correlation with difference between diameters and volumes of affected and unaffected extremities ($0.70 \geq r \geq 0.51$).

Conclusion: Our results suggest that the biceps muscle stiffness increases with increase in severity of lymphedema. The biceps muscle stiffness measured by SWE could provide a quantitative tool for following-up patients with BCRL.

Keywords: Lymphedema, elastography, breast cancer, biceps brachii

Öz

Amaç: Meme kanseri ilişkili lenfödem (MKİL) etkilenen el ve kolda şişme, ağırlık hissi, katılık, ağrı, uyuşukluk ve hareket kısıtlılığına neden olur. MKİL bu olgularda fibrozis, selülüt, lenfadenit ve sepsise yatkınlık yapar. Bu çalışmanın amacı biseps kasında "makaslama dalgası elastografi" (SWE) ile ölçülen sertlik ve lenfödem şiddeti arasındaki korelasyonu değerlendirmektir.

Yöntemler: Üst ekstremitede MKİL olan 20 olgu (ortalama yaş; 54,6±5,4 yıl) prospektif olarak çalışmaya dâhil edilmiştir. Biseps kası sertliği SWE ile değerlendirilmiştir. Her bir olguda etkilenen taraftaki biseps kasında "makaslama dalgası" hız değerleri ölçülmüştür.

Lenfödem şiddetine lenfödemden etkilenen tarafla etkilenmeyen taraf arasındaki hacim ve çap farkı hesaplanılarak karar verilmiştir. Her olguda biseps kasında "Shear Wave" hız değeri (SWH) ölçülmüştür. SWE ile ölçülen biseps kası sertliği ve etkilenen tarafla etkilenmeyen taraf arasındaki hacim ve çap farkları arasındaki korelasyon değerlendirilmiştir.

Bulgular: Etkilenen kolda biseps kasında ölçülen SWH değerleri ile etkilenen ve etkilenmeyen ekstremiteler arasındaki hacim ve çap farkı arasında orta derecede bir korelasyon saptanmıştır ($0,70 \geq r \geq 0,51$).

Sonuç: Çalışmamızda lenfödem şiddeti arttıkça biseps kası sertliğinde artış olduğunu gösterdik. SWE ile ölçülen biseps kası sertliği MKİL tanılı olgularda takipte kullanılabilecek kantitatif bir metot olabilir.

Anahtar Kelimeler: Lenfödem, elastografi, meme kanseri, biseps

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Introduction

Breast cancer-related lymphedema (BCRL) is one of the most common complications of breast cancer survivors. It occurs as a result of fluid accumulation in the interstitial tissue due to damage of the lymphatic system, induced by surgery and/or radiation, or tumor-induced neo-lymphangiogenesis [1]. BCRL causes upper limb swelling, pain, immobility and it seriously affects the quality of life [2-4].

Pathologic processes such as chronic inflammation with fibrosis, increased cellularity and desmoplastic reaction in neoplastic diseases alter tissue ultrastructure and stiffness [5]. Ultrasound (US) elastography is a non-invasive, inexpensive, and useful complementary tool that allows measuring tissue stiffness and it also improves the diagnostic performance of B-mode US [6]. Recently elastography is being used in imaging of many tissues including breast, thyroid, liver, lymph nodes and musculoskeletal tissues in clinical practice. Presently, two main US elastography techniques are used in clinical practice: strain elastography (SE) and shear wave elastography (SWE). SE uses manual compression produced by operator pressing the transducer and provides qualitative and semiquantitative analysis of the lesions. SE measures the displacement of tissue and it can be affected by the degree of compression. SWE is one of the dynamic elastography techniques which use shear waves generated by acoustic radiation force [7]. SWE enables quantitative analysis of tissue elasticity without compression and it is more objective than the SE [7, 8].

Several imaging techniques have been used for qualitative assessment of lymphedema. Lymphoscintigraphy is a standard diagnostic imaging modality in which the lymphatic system in the affected limb can be demonstrated by a radiotracer [9]. The main problem with lymphoscintigraphy is the poor spatial resolution of the images. Indocyanine green (ICG) fluorescence lymphangiography is another imaging technique using near infrared technology. ICG fluorescence lymphangiography allows evaluating the superficial lymphatics and it is a baseline imaging modality for deciding on surgical intervention. The main disadvantage of fluorescence lymphangiography is being an intraoperative procedure [10]. Contrast-enhanced MR lymphography is another invasive imaging modality and it remains as an experimental study [11]. Previously US have been shown to be a reliable imaging modality by comparing the thickness of the skin and subcutaneous tissue [12-15]. Both the skin and subcutis are thickened in the ipsilateral swollen arm compared with the contralateral arm, confirming the clinical impression [16]. After breast cancer treatment, not only the lymphatic drainage of the subcutis of the arm both also the lymphatic drainage in the muscle could be affected [17]. There are a few numbers of studies in the literature evaluating lymphedema by elastography. Elastography has been studied in differentiating between lymphedematous and normal tissues and it has been suggested as a feasible method to differentiate normal and lymphedematous tissues by measuring the stiffness of arm and leg subcutis tissue [18, 19]. We aimed to demonstrate the the effects of lymphedema on the muscle tissue. To the best of our knowledge, the stiffness of biceps muscle in patients with BCRL has not been studied yet in the literature.

Herein, we aimed to analyze the correlation between the biceps brachii muscle stiffness measured by Shear Wave Elastography (SWE) and the severity of the lymphedema.

Material and methods

Subjects

This study conforms to the Declaration of Helsinki. The study was approved by the Institutional Review Board and Ethics Committee and supported by the Institutional Research Fund (project number: KA16/150, 2016). Written informed consent was obtained from all patients.

The inclusion criteria were: having unilateral BCRL and clinically detected lymphedema (a circumferential difference of ≥2 centimeter or volumetric difference of ≥ 200 mL, between the affected and non-affected arms). The exclusion criteria were as follows: prior surgery or major trauma to the upper extremity and any other muscular disorders, bilateral breast cancer survivors. Thus, this prospective study included 20 consecutive patients with having BCRL in the upper limb.

Demographic data including age, body mass index (BMI as kg/m2), side of the dominant extremity, duration of time since the diagnosis of breast carcinoma, duration of time since swelling started, received treatment modalities were evaluated.

Assessing lymphedema

The severity of the lymphedema was determined by the difference between the circumferential diameters and volumes of the affected and unaffected extremities. Before taking volumetric and circumferential arm measurements, women were asked to remove all jewelry, compression bandages and compression sleeves from the upper extremity. Water displacement volume method is used to assess the volume of the upper limb.

A larger water container with an overflow pipe drained into a different smaller container. The larger container was filled with warm water up to a mark at the lower border of the overflow pipe. Each arm was placed in the container in turn and the volume of water displaced was recorded as mm3 (Figure 1).

A flexible, non-stretch fabric type measurement tape was used to measure arm circumferences. Measurements were performed at the level of the 10th cm proximal to the lateral epicondyle as mm.

US and SWE examinations

The US and SWE examinations were performed with a US system (Acuson S 2000; Siemens, Erlangen, Germany). SWE was performed by using a probe with an L9-4 linear array. Each patient was examined by the same radiologist (with >3 years of experience in musculoskeletal radiology). The radiologist was blinded to physician's examination.

All US and SWE examinations were performed with the patient in the sitting position in front of an examination bed with their arms extended down by their side. The arms were extended and the forearms held in the supine position. Care was taken to examine in a neutral position as much as possible. A standard US examination was initially performed to view the biceps muscle in the axial plane. Longitudinal plane was preferred for SWE measurements to visualize the muscle more widely. A rectangular electronic box-shaped region of interest (ROI) was used for shear wave speed (SWS) measurements automatically provided by the system software (Figure 2). The quality of the images was assessed by color-coded quality maps provided by the US system in which the green areas were considered reliable. The yellow and red color–coded areas were considered as low-quality scans. The scanning was repeated till high-quality images were obtained and the best representative image of the highest quality on the quality map was selected to measure SWS. The stiffness of the biceps brachii muscle was measured by virtual touch tissue imaging quantification method (VTIQ). SWS of the biceps muscle on the affected side for each patient were measured. The correlations between the biceps brachii muscle stiffness measured by SWE and the difference between the

diameters and volumes of the affected and unaffected extremities were analyzed.

Statistical analysis

Descriptive statistics were expressed as mean ± standard deviation. Kolmogorov-Smirnov test was used to test normality of data. The correlation of sonoelastographic findings with the volumes and diameters were assessed by using Pearson correlation coefficient test. The correlation coefficients were interpreted as either excellent r≥0.91; good 0.90≥r≥0.71; fair 0.70≥r≥0.51; weak 0.50≥r≥0.31; or little or none r≤0.3. The significance level was determined at p<0.05. All statistical tests were performed using IBM SPSS Statistics software program (Chicago, IL, USA) for Mac version 20.0.

Figure 1: Assesing volume of the arm by water displacement method.

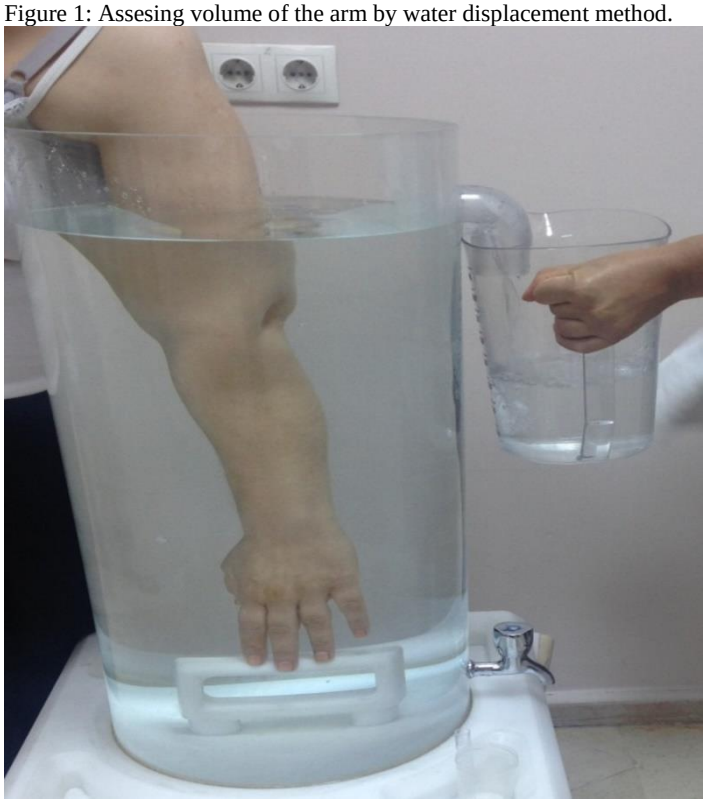
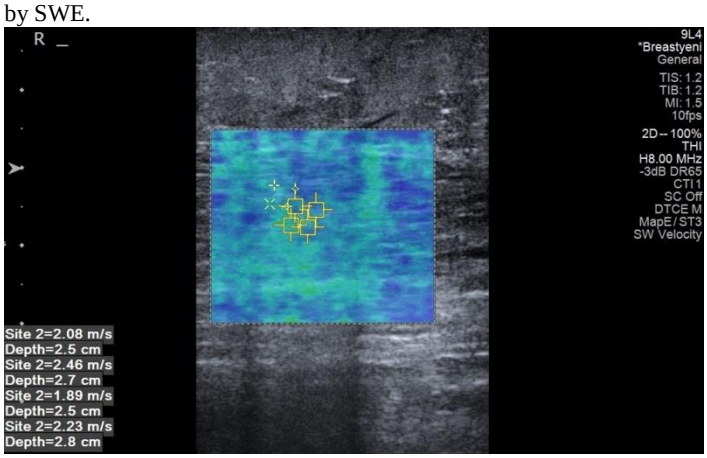


Figure 2: Shear Wave Speed (SPS) measurement of the biceps muscle by SWE.



Results

The mean age of the study population was 54.6±5.4 years. Mean BMI of the patients was 28.8 ± 20.9 kg/m2. The affected extremity was the dominant extremity in the 14 (70%) of the patients.

Duration of the time since the diagnosis of breast carcinoma was 63.9± 61.5 months, and duration of the time since swelling started ranged from 1 month to 120 months (median 12 months). All the patients had surgery and also all of them received chemotherapy. Nineteen of the patients (95%) had axillary lymph node dissection. Eighteen of the patients (90%) received radiotherapy. The detailed demographic information of the study population is demonstrated in Table.

Mean volumetric difference between the affected and unaffected extremities was 912.62±564.5 mm3. Mean circumferential measure between the diameters of the affected and unaffected extremities was 35.3±1.4 mm.

Mean SWS of the biceps muscle on the affected side was 2.27±0.40m/s. SWS of the biceps muscle on the affected side showed fair positive correlation with the difference between the volumes of the affected and unaffected extremities (r=0.65, p=0.043).

There was a fair positive correlation between the SWS of the biceps muscle on the affected side and the difference between the diameters of the affected and unaffected extremities (r=0.62, p=0.039).

Table 1: Clinical characteristics of the study population.

Characteristics	
Age (years)[¥]	54.6±5.4
Body mass index (kg/m^2)[¥]	28.8±20.9
Duration of time since breast carcinoma diagnosed (months)[¥]	63.9±61.5
Location of tumor[µ]	
Upper outer quadrant	14
Upper inner quadrant	3
Lower outer quadrant	2
Lower inner quadrant	1
Type of breast surgery[µ]	
Modified radical mastectomy	12
Breast conserving surgery	8
Type of axillary lymph node surgery[µ]	
Sentinel lymph node biopsy	19
Level I-II axillary lymph node dissection	17
Radiotherapy[µ]	19
Chemotheraphy[µ]	20
Duration of lymphedema (months)[β]	12 (1-120)

[¥]: mean±standard deviation, [µ]: n, [β]: median (range)

Discussion

This study showed an association between the severity of lymphedema and the stiffness of the biceps muscle. Quantification of lymphedema by measuring limb size or limb volume has been used in both clinical practice and research studies. Among BCRL patients, shoulder and arm diseases including rotator cuff disease, adhesive capsulitis and axillary web syndrome can be seen and these diseases affect the morbidity of both shoulder and arm. Also tendon of the long head of the biceps can be affected in BCRL [20, 21]. Previously, Jang et al. showed bursal thickening and distension of the biceps brachii tendon sheeth among patients with BCRL. Jang et al found that the patients with a supraspinatus tendon tear had a significantly longer duration of lymphedema [21]. After breast cancer treatment the lymphatic drainage of the muscle could be affected [17]. We aimed to detect the possible effects of BCRL on the muscle stiffness. The biceps muscle was chosen for this goal because it was relatively superficially located and less affected from the underlying bone artefacts. This is the first study in the literature assessing the stiffness of biceps muscle among the patients with BCRL.

Volumetric and circumferential measurement methods are commonly used to monitor lymphedema [22]. However, the early stage of lymphedema may exist months or years before swelling occurs [23-25]. Previously, minimal limb volume change has been shown to have a significant impact on breast

cancer survivors [26]. New assessment tools are needed to early diagnose the lymphedema before swelling of the limb. SWE may provide additional quantitative information in early diagnosis of lymphedema. Further studies are needed to determine SWE's reliability, sensitivity, and specificity in early diagnose of lymphedema.

The patients were over-weighted in our study population. We proposed that obesity could be a factor increasing the predisposition to lymphedema. Our results corresponded with a recent study that showed a significant correlation with increased arm edema and abdominal obesity among the patient with BCRL. Increased BMI should be considered as an aggravating factor for lymphedema severity [27]. Overweight and obese women with BCRL are more likely to have increased abdominal fat than the general population and obesity has been already defined as an increased risk factor for the development of BCRL [28, 29].Water displacement method has been considered to be the "gold standard" for measuring the lymphedema [30]. Considering the difficulties associated with water displacement volumetric method, an alternative method is needed. SWE could be a fast and practical alternative method.

Our study showed that the stiffness of the biceps muscle increases as the severity of lymphedema increases. The main factor increasing the muscle stiffness in severe lymphedema could be the increased pressure among the soft tissues. This pressure may result with compression to the muscles. Histologically, compression may cause acutely edema but chronic compression may progress to fibrosis. SWE results may reflect the structural changes in muscles.

Although swelling and increased volume of the upper limb are the main complaints of women with BCRL, pain and firmness are the other subjective findings [31]. The goal of the treatment modalities in BCRL includes decreasing not only the amount of swelling in the upper limb, but also reducing the pain and firmness. We suggest that SWE could be an objective modality also in monitoring the patients with lymphedema.

A potential limitation of our study was not calculating intra- and inter-rater reliability of the measurements. Other limitation of our study was that the stiffness of the contralateral biceps muscle was not measured. At the beginning of the study, we hypothesized only evaluating the affected extremity. However, it would be better to compare the stiffness of the biceps muscle on the affected and unaffected extremities. The detailed data about TNM stage, radiotherapy protocols with regard to the dose, area and duration could not be collected for all patients. This would be a potential limitation of our study.

In conclusion, BCRL is a chronic and debilitating disease and it might be generally under-diagnosed and undertreated. The effects of lymphedema on a patient's quality of life can be devastating. In conclusion, we suggest that the biceps muscle stiffness measured by SWE could provide a quantitative tool in early detection of lymphedema and might be helpful in objective monitoring lymphedema during follow-up in patients with BCRL.

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A new nonsurgical experimental model for Asherman syndrome created in rats

Sıçanlarda oluşturulan cerrahi müdahalesiz yeni bir deneysel Asherman sendromu modeli

Başak Büyük [1]

Abstract

Aim: Asherman Syndrome (AS) is a partial or complete obstruction of the uterine cavity with adhesions as a result of trauma. In pre-clinical studies, to be able to show the effectiveness of new treatment methods, first of all, the AS model needs to be created in the animals. The aim of this study is to develop a new and effective nonsurgical method for using in AS and intrauterine adhesions modeling, and through this way, to propose a more effective method for researchers in terms of safety and feasibility.

Methods: Twelve female Wistar Albino rats were divided into two groups. It was reached to the left uterine horn transvaginally by using pre-prepared pink color (20 gauge) cannula. While 0.2 ml normal saline was applied to the animals in Group I (control group), 0.2 ml (Trichloroacetic acid) TCA was applied to the animals in Group II (experiment group).The right uterine horns of the animals were left without treatment. After three menstrual cycles, the animals were sacrificed and Hematoxylin-Eosin and Masson's Trichrom stainings were performed and evaluated histopathologically. Inflammation was evaluated in Hematoxylin-Eosin staining and fibrosis was evaluated in Masson's Trichrom staining.

Results: Whereas the uterine sections of the Group I have normal histologic appearance, inflammation and fibrosis were found in the left uterine sections of the Group 2 by histopathological evaluation. Results were statistically significant (p=0.002).

Conclusion: The proposed nonsurgical AS modeling method created disease, and this was also revealed by histopathological examinations. Through this way, a new AS model is suggested without surgery, in which the disease is correctly created.

Key words: Asherman syndrome, rat, gynatresia, pathology, animal model.

Öz

Amaç: Asherman sendromu(AS) uterin kaviteye travma sonucu kavitenin adezyonlarla kısmi ya da tam tıkanması durumudur. Klinik öncesi çalışmalarla yeni tedavi yöntemlerinin etkinliğini gösterebilmek için öncelikle AS modelinin hayvanda oluşturulmasına ihtiyaç vardır. Bu çalışmanın amacı, AS ve intrauterin adezyon modellemesinde kullanılmak üzere cerrahi uygulanmaksızın yeni ve etkili bir yöntem geliştirmek, bu şekilde araştırmacılara güvenlik ve uygulanabilirlik açısından daha etkin bir metot önerebilmektir.

Yöntem: On iki adet dişi Wistar Albino sıçan rastgele 2 gruba ayrıldı. Daha önceden hazırlanmış olan pembe renk (20 gauge) kateterle transvajinal yoldan hayvanların sol uterin hornularına ulaşıldı. Grup I'deki hayvanlara 0,2 ml serum fizyolojik uygulandı, Grup II'dekilere ise 0,2 ml Triklorasetik asit (TCA) uygulandı. Üç menstruel siklus beklendikten sonra hayvanların uterusları çıkarılıp Hematoksilen-Eozin ve Masson Trikrom boyamalar yapılarak histopatolojik olarak değerlendirildi. Yapılan Hematoksilen-Eozin boyalı kesitlerde inflamasyon dereceleri değerlendirilirken, Masson Trikrom boyalı kesitlerde fibrozis dereceleri değerlendirildi.

Bulgular: Grup 1'e ait uterus kesitleri normal histolojik görünüme sahipken, Grup 2'ye ait sol uterus kesitlerinde inflamasyon ve fibrozis oluştuğu gözlendi. Bu sonuçlar istatistiksel olarak anlamlıydı (p=0,002).

Sonuç: Sonuç olarak, önerdiğimiz cerrahi müdahalesiz AS modelleme yöntemi, literatürde önerilen yöntemler ile benzer şekilde hastalık oluşturmuş ve bu etki, yapılan histopatolojik incelemelerle de ortaya konmuştur. Bu şekilde, AS için doğru bir hastalık modellemesi gerçekleştiren cerrahisiz yeni bir yöntem önerilmiştir.

Anahtar kelimeler: Asherman sendromu, sıçan, jinatrezi, patoloji, hayvan modeli

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Ethics Committee Approval: The study was approved by the local ethical authority.
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Introduction

Asherman Syndrome (AS) is a partial or complete obstruction of the uterine cavity with adhesions as a result of trauma [1]. In clinical practice, AS may cause menstrual disorders, such as amenorrhea, dysmenorrhea, or hypomenorrhea, infertility, recurrent pregnancy losses, and placental disorders [2, 3]. In these patients, endometrial biopsies often give the appearance of postmenopausal endometrium. Looking at the pathology of intrauterine adhesions (IUA), it can be seen that it has a fibrous structure. The IUAs, which have a feature of fibrous tissue, may occlude the uterine cavity partially or totally. Clinical symptoms also occur according to the severity of these IUAs [4]. For women with normal menstruation and reproductive characteristics, the prevalence of AS was reported between 2.8% and 45.5% [3].

Today, development of a standard treatment model for AS is a condition that researchers are studying on. Although there are different treatment options for AS, standard treatment procedure has not been developed, so 100% success has not been achieved yet [1-3, 5-9].

In addition to this protocols, stem cell treatments have been also being tested in the literature for AS, but there isn't any protocol used in clinic applications yet [10-16]. In order to further development of these studies and to pass to the clinical application, more studies on animals are needed.

To be able to propose new treatment methods for AS, pre-clinical studies should be completed. In order to demonstrate the effectiveness of the treatment methods in pre-clinical studies, firstly, the AS model needs to be created in animal. After AS modeling is carried out in experimental animals, the effectiveness of the proposed method can be demonstrated by comparing with control groups.

There are various methods in the literature for accurate and effective modeling of AS in experimental animals. In all of these cases, under anesthesia, the uterine cavity is reached by opening the abdomen via abdominal incision, then, chemical, physical or infectious trauma is created and the uterus and the abdominal cavity are closed surgically again [10, 11, 17-29]. After enough time is waited for the development of fibrosis and IUAs, the experiment is terminated. However, when the abdomen is opened by surgical methods, the animals are exposed to the risk of infections, and if the wound care is not done correctly, especially rodent-group animals can damage themselves through their wounded areas. In addition, the anesthesia and surgical process are very difficult and time consuming operations for researchers, as well as they increase the costs.

The aim of this study was to develop a new and effective method for AS and IUA modeling without applying surgical techniques, and through this way, to be able to propose an effective method in terms of safety and feasibility.

Material and methods

Materials and experimental design

The study was approved by the Institutional Animal Use and Care Committee of Canakkale Onsekiz Mart University (COMU) with No: 2017/12-07 and performed in accordance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26 and with the Helsinki Declaration of World Medical Association recommendations on animal studies. Wistar albino rats were obtained from COMU Experimental Research Application and Research Center. Twelve female Wistar Albino rats were used in the study, with a mean age of four months and weight of 240–300 g. The animals were housed in stainless steel cages in an animal room maintained at a standard humidity (45%-50%) and temperature 22±2°C with 12 hours light periods (12 hours of daylight/12 hours of dark). All animals were fed standard food and water and twelve hours before the study procedure feeding was stopped and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

Experimental procedure

Before starting the study, 20 gauge cannula was taken, its metallic needle was removed and flexible tip of the cannula was bended to the left. Twelve female Wistar Albino rats were divided into two groups;

Group I: (Normal Saline (NS) group, n=6)

Group II: (Trichloroacetic acid (TCA) group, n=6).

After the anesthetization of the animals by giving 50 mg/kg ketamine hydrochloride (Ketalar®, Pfizer İlaçları Ltd, Şti, İstanbul, Türkiye) and 10 mg/kg xylazine (Alfazyne %2, Ege Vet San. Tic, İzmir, Türkiye) to them, it was reached to the left uterine horn through transvaginal route by using pre-prepared pink color (20 gauge) cannula. While 0.2 ml NS was applied to the animals in Group I, 0.2 ml TCA (IL-33, İstanbul İlaç San. Tic. AŞ, İstanbul, Türkiye) was applied to the animals in Group II (Figure 1). For both groups, the right uterine horn was left without treatment. After waiting three menstrual cycles, the uterus of the animals were removed and histopathologically evaluated.

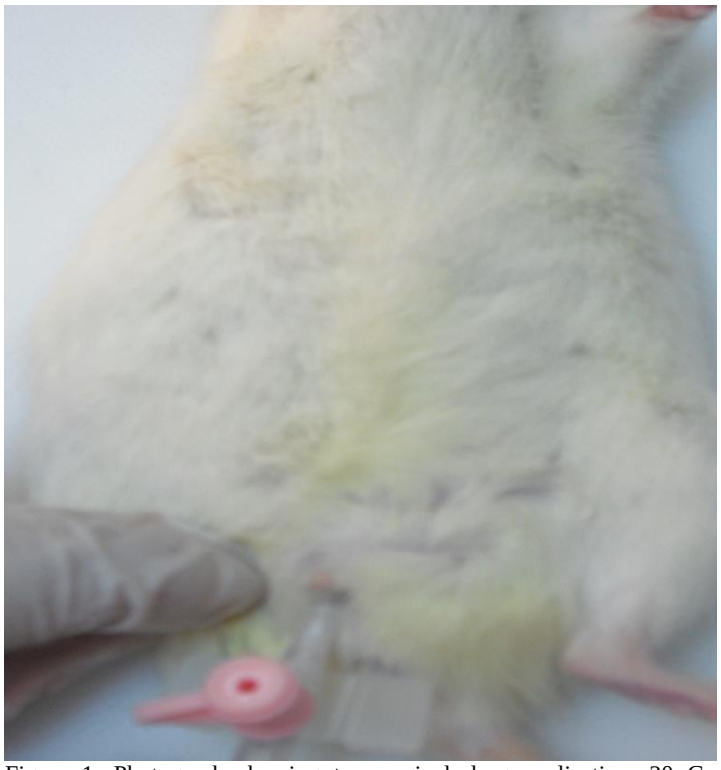

Figure 1. Photograph showing transvaginal drug application. 20 G cannula is used for reaching the uterine cavity via transvaginally.

Histopathological examinations

To investigate histopathologic changes, uterine tissue samples were consecutively numbered and placed in 10% neutral buffered formalin. After fixation with formaline, uterine tissues were embedded in paraffin. The paraffin blocks were cut in 5 mm thickness on Rotary Microtome (Leica RM2125 RTS) and the sections were stained with hematoxylin and eosin (H&E) and Masson's Trichrome methods. The histopathological sections were examined under a light microscope (Zeiss AxioScope A1) for the presence of fibrosis, inflammation, then rated on a modified semi-quantative scale of 0-3[14]. Simple columnar

epithelium-covered endometrium is considered normal if it consists of a basal layer adjacent to myometrium and a functional layer with a spongiform lamina propria, including spiral arteries and uterine glands (30). Evaluation of the specimens was done by a histologist who was blind to the study groups.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 19.0 (Chicago Inc., Chicago, IL). All continuous variables were expressed as mean ±standard deviation (SD) and median (minimum–maximum). Because of the small sample size and non-normal distribution of the data non-parametric tests were used to evaluate the results. The mean values were compared by Kruskall–Wallis and Mann–Whitney U tests. A p value less than 0.05 were considered as statistically significant.

Results

As a result of the evaluation of H&E stained sections of the tissues of animals in each group, it was observed that the right uterine horns had a normal histological appearance. While the endometrial glands and the epithelial structures were evaluated as normal, there was no finding encountered in the myometrium. The left uterine horns belonging to the animals in Group 1 also had a normal microscopic appearance. In the sections of the left uterine horns taken from the animals in Group 2, it was also microscopically observed that IUAs had been developed in all animals' sections (Figure 2). In the Masson's Trichrome staining, the levels of fibrosis were evaluated (Figure 3). In animals of group 2, while IUAs were observed in the tissue sections of the left uterine horn, the fibrosis and inflammation scores were determined as grade 3 (Figure 4). Inflammation and fibrosis scores of group 2 are significantly higher than group 1 (p=0.002). No fibrosis was observed in the uterine horns of group 1 animals and the right horns of group 2 animals.

Table 1. Comparison of group 1 and group 2 in terms of inflammation.

	Group 1 (n=6)	Group 2 (n=6)	
Grade	n(%)	n(%)	p
0	5 (83.33)	0 (0)	0.002
1	1 (16.66)	0 (0)	
2	0 (0)	2 (33.33)	
3	0 (0)	4 (66.66)	

Table 2. Comparison of group 1 and group 2 in terms of fibrosis

	Group 1 (n=6)	Group 2 (n=6)	
Grade	n(%)	n(%)	p
0	6 (100.00)	0 (0)	0.002
1	0 (0)	0 (0)	
2	0 (0)	2 (33.33)	
3	0 (0)	4 (66.66)	

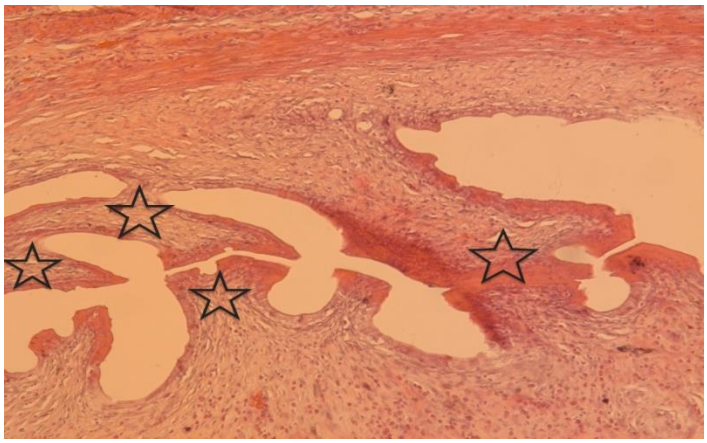

Figure 2. Intrauterine adhesions are seen in sections belong to the left uterine horn in Group 2 Stars show fibrotic bands which generate IUAs (H-E staining x100 magnification).

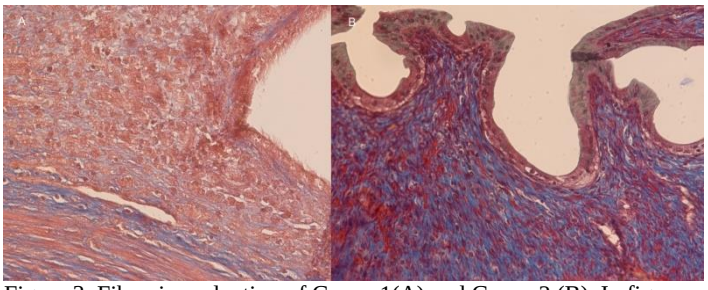

Figure 3. Fibrosis evaluation of Group 1(A) and Group 2 (B). In figure A, no fibrosis is seen. In figure B, fibrosis is seen in dark blue coloured areas (Masson's Trichrome staining x200 magnification).

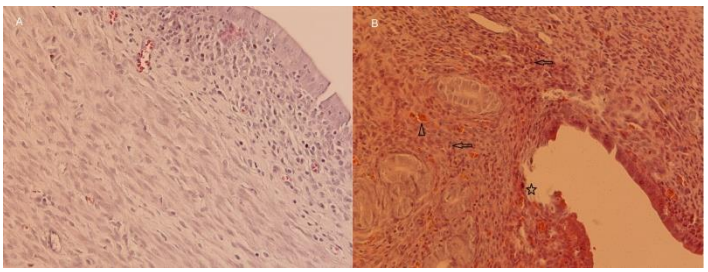

Figure 4. Histopathological evaluation of Group 1(A) and Group 2 (B). Inflammation is seen in figure B. Arrows show inflammatory cells, arrowhead shows vascular congesion and star shows epithelial erosion (H-E staining x200 magnification).

Discussion

AS is a condition that develops as a result of endometrial trauma, followed by IUAs, and causes clinical results such as menstrual disorders and infertility [31-33]. Pathological endometrial thinning is the result of damage to the basal layer, which results in the failure of the functional layer proliferation [2].

Today, development of a standard treatment model for AS is a condition that researchers are studying on. There are a number of methods proposed for the AS modeling in the literature which AS modeling was carried out by endometrial scratching [10, 11, 21, 23, 25, 26]. In some of these studies, it is seen that additional procedures are needed to create endometrial damage that is adequate to create IUA [25]. Especially in the AS modeling method recommended for the rabbits, scratching was performed with a 4 mm endometrial curette and it was attempted to create an infection by using infectious agents. It has been reported that a better result was achieved by the binary method like this way [25]. In all these studies, operations were performed by laparatomy under anesthesia. At the end of the waiting period determined for modeling process, the abdomen was opened again and tissue samples were taken. All of these surgical procedures can make the researchers to loss both time and money. Moreover, because of the importance of the care of animals after the surgery, additional workload emerges. In addition, if appropriate care is not given after surgery, animal deaths can be too great and the experiment can be unsuccessful.

In other studies suggested in the literature for the formation of IUA and AS models, endometrial trauma was attempted to create by using 24 G [18] or 27 G [16, 19, 20] needles. In those studies, the abdomen was opened surgically, the uterine horns were reached and trauma was created by entering the lumen with a needle. Since those studies also require surgery, they can bring additional load in terms of time and cost. After the operation, animal care and especially the wound follow-up may be trouble again and require being more careful. Moreover, if surgery is required for the treatment method to be tried after the modeling, laparotomy may be needed several times and may cause negative results for wound healing. Therefore, animal

follow-up and care are easier in our model that can be done non-surgically.

In addition to physical trauma, in the literature, there are also some studies in which AS is created chemically [17, 22, 24, 29]. In those studies, the experimental procedure is almost the same as in the previous ones; under anesthesia, the abdomen is opened and the related uterine horn is reached. With ethanol or TCA injection into the uterine horn, endometrial damage is attempted to create. All the side effects of the surgery are also valid for those studies, and animal care processes still needs special interest.

In our proposed modeling, the uterine cavity is reached through transvaginal route in the rats, with the TCA injection [14] used earlier in the literature, endometrial trauma is created, and after waiting enough time to prevent the fluid from escaping back, the catheter is withdrawn. In this model, it was microscopically shown that all pathological features of the AS were obtained. In addition, after the operation, no problems were observed in the follow-up process of animals and no special care was required.

In conclusion, similar to the methods suggested in the literature, the proposed nonsurgical AS modeling method created disease, and this effect was also revealed by histopathological examinations. Through this way, a new AS model is suggested without surgery, in which the disease is correctly created.

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Comparison of three different creatinine clearance calculation methods in patients with type 2 diabetes mellitus

Tip 2 diyabetik bireylerde kreatinin klirensini hesaplamada kullanılan üç farklı yöntemin karşılaştırılması

Fatih Orkun Kundaktepe [1], Mustafa Genco Erdem [2], Şerife Ayşen Helvacı [3]

Abstract

Aim: To determine the most accurate and useful method for calculating creatinine clearance by comparing the results of different methods.

Methods: Type 2 Diabetic 100 patients who have been followed by Okmeydani Training and Research Hospital internal medicine and/or diabetes policlinics. Individuals with Type 1 Diabetes Mellitus and acute kidney disease were excluded from the study.

Results: Glomerular filtration rate calculated with Cockroft-Gault formula was significantly affected by creatinine, weight and age ($p<0.05$ for all) in a univariate model. In a multivariate model this was significantly independently affected by creatinine, weight and age ($p<0.05$ for all). Glomerular filtration rate measured with Modification of Diet in Renal Disease formula was significantly affected by creatinine and age ($p<0.05$ for all) and in a univariate model. In a multivariate model this was significantly independently affected by creatinine ($p<0.05$). Glomerular filtration rate measured with 24h urine was significantly affected by creatinine, weight and age ($p<0.05$ for all). In a multivariate model this was significantly independently affected by weight ($p<0.05$).

Conclusion: In this study, those three methods were similar and positively correlated to each other. Such findings prove that those three different methods are compatible with each other at glomerular filtration calculation and they are all useful in clinical practice. Practical and accurately intensive follow up of those patients will give a chance of better understanding this process and will help us with intervention as soon as possible when needed.

Keywords: Glomerular filtration rate, Cockroft-Gault, MDRD, creatinine clearance.

Öz

Amaç: Diyabetik bireyler için kullanılabilecek en uygun kreatinin klirensi hesaplama metodunu belirlemek amaçlandı.

Yöntem: Çalışmaya Okmeydanı Eğitim Araştırma Hastanesi iç hastalıkları ve diyabet polikliniklerine başvurmuş 100 tip 2 diyabetik hasta dahil edildi. Tip 1 diyabet, hipertansiyon ve akut böbrek yetersizliği tanısı almış diyabetik hastalar çalışma dışı bırakıldı.

Bulgular: Cockroft-Gault değerini kestirmede tek değişkenli modelde yaş, ağırlık, kreatininin anlamlı (hepsi için $p<0,05$) etkisi gözlenmiştir. Çok değişkenli modelde ise yaş, ağırlık, kreatinin değerinin anlamlı bağımsız (hepsi için $p<0,05$) etkisi gözlenmiştir.

MDRD değerini kestirmede tek değişkenli modelde yaş, kreatininin anlamlı ($p<0,05$) etkisi gözlenmiştir. Çok değişkenli modelde ise kreatinin değerinin anlamlı bağımsız ($p<0,05$) etkisi gözlenmiştir.

24 saatlik idrarda kreatinin klirensi değerini kestirmede tek değişkenli modelde yaş, ağırlık, kreatinin değerinin anlamlı (hepsi için $p<0,05$) etkisi gözlenmiştir. Çok değişkenli modelde ise ağırlık değerinin anlamlı bağımsız ($p<0,05$) etkisi gözlenmiştir.

Sonuç: Bu çalışmada, bu üç yöntem birbirleriyle pozitif korelasyon gösterdi. Bundan yola çıkarak klinik pratikte her üç metodun da kullanılabileceği söylenebilir. Bu hastaların yakından düzenli takibi bu sürecin daha iyi anlaşılmasını sağlayacağı gibi bizlere de ihtiyaç olduğunda erken müdahale imkanı sunar.

Anahtar kelimeler: Glomerular filtrasyon hızı, Cockroft-Gault, MDRD, kreatinin klirensi

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Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır.

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Introduction

Diabetes mellitus (DM) is a chronical and progressive disease. Approximately 150 million people are suffering from this disease and predicted the number for 2025 is 300 million [1, 2].

Morbidity and mortality due to DM and its complications are increasing as the prevalence of type II DM increases [3]. Consequently, early diagnosis and effective treatment of type II DM is needed more and more every day. There are approximately 2.6 million type II DM patients in our country, and it is predicted that at least one-third of 1.8 million people still in impaired glucose tolerance stage will join to this group in the near future [4].

Diabetic nephropathy (DN) is a serious health problem causing end-stage renal failure. In the United States of America, DN causes 40 % of newly developed end-stage renal failure. DN defined as positive urine albumin stick test or excretion of albumin more than 300 mg in a diabetic patient who is not suffering from other renal diseases. DN, as appears a late finding of diabetes has some physiological, pathological and clinical symptoms. That made some researchers consider DN into stages [5].

Creatinine clearance measurement is the most common method for evaluating renal functions. Creatinine clearance may be measured with 24-hour urine collection and also with Cockcroft-Gault formula and MDRD.

In this study, we aimed to to determine the most accurate and useful method for calculating creatinine clearance by comparing the results of different methods. It was aimed to improve feasibility by determining the most suitable method to be possible.

Material and methods

This retrospective study approved by Okmeydani Training and Research Hospital Clinical Research Ethical Board Presidency with a number of 178 at 09.09.2014. Files of type 2 DM patients who applied to one of internal medicine outpatient clinics between 2012 and 2014 were retrospectively screened. From a total of 184 patients; patients with hypertension (n=74), acute renal failure (n=6) or renal transplantion (n=4) were excluded from the study. The remaining 100 patients (56 female, 44 male) included to the case group. Median age of the patients was 56 years with a range from 20 to 82 years.

Patients' characteristics (age, gender and weight (kilograms)) and laboratory findings (serum creatinine level (mg/dl), fasting blood glucose (mg/dl), postprandial blood glucose (mg/dl), HbA1c (%) and 24-hour urine creatinine clearance (GFR24) (mg/24 hours)) were evaluated. Roche-Hitachi Cobas 8000 (Serial number: 1349-09, 2014, Japan) was used to evaluate laboratory findings. The prediction of creatinine clearance (in ml/min) by the Cockcroft-Gault formula (GFRC&G) was calculated as (140 − age) × body weight/plasma creatinine × 72 (× 0.85 if female) [6]. The abbreviated MDRD (GFRMDRD) estimate of the kidney function was calculated as 175 × plasma creatinine−1.154 × age−0.203 (× 0.742 if female) [7]. Grading of the patients with regard to renal failure were performed according to the KDIGO 2017 guideline using GFR values (G1-G5) (Table 1) [8].

Statistical analysis

IBM SPSS for Windows 21.0 (Armonk, New York, USA) statistics package program was used. Mean, median, minimum, maximum, frequency values and standard deviation were used for defining statistics of data. Distribution of the variables was controlled with Kolmogorov Simirnov test. Unpaired t-test and Mann-Whitney U test were used for quantitative data analysis. Chi-square test was used for qualitative data analysis. Spearman correlation test was used for correlation analysis. Univariate and multivariate regression tests were performed. Level of significance was determined as $p \leq 0.050$ for all.

Table 1. Glomerular filtration rate categories in chronic renal failure*.

GFR category	GFR (ml/min/1.73m^2)
G1	≥ 90
G2	60 - 89
G3a	45 - 59
G3b	30 - 44
G4	15 – 29
G5	< 15

* In the absence of evidence of kidney damage, neither category G1 nor G2 fulfill the criteria for chronic renal failure.

Results

A total of 100 patients were staged by GFR. Sixty-nine patients (69%) had GFR greater than 90 mL/min. staged as G1, 22 patients (22%) had GFR between 60-89 mL/min staged as G2 and 9 patients (9%) had GFR between 30-59 mL/min staged as G3. None of the patients staged as G4 and G5.

Creatinine clearance of the patients was calculated by Cockcroft-Gault formula (GFRC&G), Modification of Diet in Renal Disease (GFRMDRD) and 24h urine collection method (GFR24). Mean values of these three methods were 96.4±28.8 mL/min, 104.5±29.8 mL/min and 86.2±24.7 mL/min for GFR24, GFRC&G and GFRMDRD, respectively.

Table 2 shows statistical values of patients' in terms of gender, weight, fasting blood glucose, postprandial blood glucose, HbA1c, GFR24, GFRC&G and GFRMDRD (Table 2).

Table 2. Characteristic features of the patients.

Variable	
Age (years)¥	56.1±10.2
Genderβ	
Female	56 (56)
Male	44 (44)
Weight (kg)¥	84.5±14
Creatinine (mg/dL)¥	0.9±0.3
Fasting blood glucose (mg/dL)¥	175.7±64.7
Postprandial blood glucose (mg/dL)¥	258.3±97.4
HbA1c (%)¥	8.2±1.8
Cockcroft-Gault (mL/min)¥	104.5±29.8
MDRD (mL/min/1.73m^2)¥	86.2±24.7
Creatinine Clearance with 24h urine (mL/min)¥	96.4±28.8

¥: mean±standard deviation, β:n (%)

Significant ($p<0.050$ for all) negative correlation was observed between creatinine levels and GFRC&G, GFRMDRD, GFR24. Significant ($p<0.050$ for all) positive correlation was observed between GFRC&G, GFRMDRD and GFR24. Significant ($p<0.050$ for all) positive correlation revealed between fasting blood glucose, postprandial blood glucose and HbA1c (Table 3).

In both univariate and multivariate models age, weight, and creatinine had significant ($p<0.050$ for all) association on determining GFRC&G value (Table 4). Although in a univariate model age and creatinine had significant ($p<0.050$ for all)

association on determining GFRMDRD value; in a multivariate model only creatinine had independently significant (p=0.001) association (Table 5). Although in a univariate model age, weight and creatinine had significant (p<0.050 for all) association on determining GFR24 value; in a multivariate model only weight had independently significant (p=0.001) association on determining GFR24 value (Table 6).

		Creatinine (mg/dL)	Fasting blood glucose (mg/dL)	Post prandial blood glucose (mg/dL)	HbA1c (%)	Cockcroft-Gault (mL/min)	MDRD (mL/min/ 1.73m^2)
Fasting Blood Glucose (mg/dL)	r	0.145					
	p	0.149					
Postprandial Blood Glucose (mg/dL)	r	0.096	0.633				
	p	0.340	0.001				
HbA1c (%)	r	0.081	0.702	0.504			
	p	0.423	0.001	0.000			
Cockcroft-Gault (mL/min)	r	-0.373	0.086	-0.004	0.187		
	p	0.001	0.392	0.968	0.062		
MDRD (mL/min/1.73m^2)	r	-0.592	-0.111	-0.109	-0.029	0.660	
	p	0.001	0.273	0.280	0.775	0.001	
Creatinine Clearance with 24h urine (mL/min)	r	-0.399	-0.137	-0.135	-0.057	0.679	0.627
	p	0.001	0.175	0.181	0.572	0.001	0.001

Table 3: Correlations with different methods calculating glomerular filtration rate.

Cockcroft-Gault	Univariate model				Multivariate model			
	β	95.0% CI		p	β	95.0% CI		p
		Low	High			Low	High	
Age (years)	-1.74	-2.21	-1.28	0.001	-1.08	-1.24	-0.9	0.001
Gender	10.69	-1.09	22.46	0.075				
Weight (kg)	1.19	0.84	1.54	0.001	1.12	1.00	1.23	0.001
Creatinine (mg/dL)	-51.8	-67.00	-36.77	0.001	-35.12	-41.74	-28.50	0.001
Fasting glucose (mg/dL)	0.00	-0.09	0.10	0.931				
Postprandial glucose (mg/dL)	0.00	-0.06	0.06	0.984				
HbA1c (%)	2.03	-1.20	5.25	0.215				

Table 4: Evaluation of the Cockroft-Gault method by linear regression.

MDRD	Univariate model				Multivariate model			
	β	95.0% CI		p	β	95.0% CI		p
		Low	High			Low	High	
Age(years)	-0.91	-1.36	-0.46	0.001				
Gender	9.61	-0.14	19.36	0.053				
Weight(kg)	0.18	-0.17	0.53	0.311				
Creatinine (mg/dL)	-49.92	-61.4	-38.43	0.001	-26.20	-37.01	-15.4	0.001
Fasting glucose (mg/dL)	-0.06	-0.14	0.01	0.111				
Postprandial glucose (mg/dL)	-0.03	-0.08	0.02	0.237				
HbA1c (%)	-0.37	-3.07	2.32	0.784				

Table5. Evaluation of the MDRD method by linear regression.

Discussion

The incidence of DN is increasing in proportion to DM incidence and increased lifetime in diabetics. Our study showed that 73% of patients had GFR under 120 mL/min. However, in our study, there was no significant correlation between fasting blood glucose, postprandial blood glucose and HbA1c and GFR values measured by three different methods.

24h urine creatinine clearance	Univariate Model				Multivariate Model			
	β	95.0% CI		p	β	95.0% CI		p
		Low	High			Low	High	
Age (years)	-1.02	-1.54	-0.49	0.001				
Gender	8.74	-2.69	20.16	0.133				
Weight (kg)	0.82	0.44	1.20	0.001	0.42	0.06	0.8	0.024
Creatinine (mg/dL)	-40.02	-55.82	-24.21	0.001				
Fasting glucose (mg/dL)	-0.08	-0.17	0.01	0.080				
Postprandial glucose (mg/dL)	-0.04	-0.10	0.02	0.199				
HbA1c (%)	-1.09	-4.22	2.04	0.491				

Table 6. Evaluation of 24h urine creatinine clearance method by linear regression.

This study compared the most popular three methods for calculating creatinine clearance. One of those methods, Cockcroft & Gault formulation uses serum creatinine, age, weight, and gender to calculate creatinine clearance by the unit ml/min [6].The second one is MDRD formulation uses race, age, serum creatinine and gender [7]. The last method is to evaluate the creatinine level in urine patient collected for 24 hours without interruption.

A study compared Cockcroft & Gault formulation, and MDRD formulation suggested that Cockcroft & Gault formulation calculated the lowest creatinine clearance in patients above age 70; while MDRD formulation is the most valuable method to estimate mortality rate in patients above age 85 [9]. In this study, the median age was under 70. GFRMDRD was slightly lower than GFRC&G without statistical signification. Yet another study published in 2007 suggested that Cockcroft&Gault formulation achieved more accurate results than other methods [10]. Another study published in 2010 suggested that Cockcroft&Gault formulation is superior to the MDRD formulation in patients with normal creatinine clearance and diabetics with normal or close to normal GFR. Otherwise, MDRD formulation has had more accurate results [11].

Our study revealed serum creatinine levels are increasing with age. Yet increased age resulted with lower GFRC&G, GFRMDRD, and GFR24h. Those results pointed out that age may be a prognostic factor for diabetic nephropathy. A study published at 2002 including 98.688 patients age between 20 and 94 years showed progressively increasing serum creatinine levels in male patients from age 60 and female patients from age 40 [12]; results of our study are consistent with this study.

In our study, independent factors that significantly affected GFRC&G increase are age, weight and serum creatinine. This result was expected as they all are variables in the Cockroft-Gault formulation. This result is consistent with the findings of two other studies. [13, 14]; and being in association with weight, is seemed to be the weakness of Cockroft-Gault formulation. Because of this deficit, another study recommended of estimating a CrCl range with the lower boundary defined by using ideal body weight in the Cockcroft-Gault equation and the upper boundary by using total body weight [15].

Independent factors significantly affected GFRMDRD increase are age and serum creatinine. This is consistent with the

previous studies [13, 16]. This result was expected as they are also variables in the MDRD formulation. It is not surprising that there is no effect of weight on the GFRMDRD since the MDRD formula does not use weight.

Independent factors significantly affected GFR24h increase are age, weight, and serum creatinine. As creatinine is released from the muscles and muscles are the big part of our weight; weight should be considered normal to affect the GFR24h.

An increase in GFR24h had a positive correlation with GFRMDRD and GFRC&G. This result indicated these three methods are consistent among themselves.

Major limitations of this study are being retrospective and the small sample size: Because of the retrospective design of the study some important clinical features could not be recorded. The small sample size may have limited our ability to detect statistically significant results.

In conclusion, there was no statistically significant difference between Cockcroft-Gault formulation, MDRD formulation and creatinine clearance with 24 hours urine method; they are all equally useful in clinical practice. So all of three methods can be used for evaluating renal functions in Type II diabetic patients but creatinine clearance with 24 hours urine method requires two patient visits in a row and a more complex biochemistry laboratory, and it might give incorrect results because of lack of communication between physician-patient-laboratory triangles especially in an outpatient clinic. In our opinion, this method may remain in the background because of the process.

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Comparison of efficiency of serratus anterior plane block and thoracic epidural block for thoracotomy analgesia

Torakotomi analjezisi için uygulanan serratus anterior alan ve torakal epidural blok etkinliklerinin karşılaştırılması

Korgün Ökmen [1]

Abstract

Aim: Currently, regional anesthesia methods are frequently used for post-thoracotomy pain. In this study, we aimed to compare the efficacy of serratus anterior plane block and thoracic epidural block for pain after thoracotomy.

Methods: This retrospective study included 120 patients who underwent thoracotomy. Seventy patients who met the inclusion criteria were evaluated in two groups as Group E (thoracic epidural block) (n=37) and Group S (serratus anterior plane block) (n=33). Postoperative 2nd, 6th, 12th and 24 hour visual analogue scale scores and total analgesic consumption for 24 hours were evaluated. Secondary outcomes were determined as side effects, additional analgesic drug requirement and complications.

Results: In the comparison between the groups, there were no statistically significant differences between two groups in terms of postoperative 2nd (p=0.417), 6th (p=0.271), 12th (p=0.734) and 24th hour (p=0.157) visual analogue scale scores and the amount of total analgesic consumption for 24 hours (p=0.714). There was no statistically significant difference between two groups with regard to the side effects nausea and vomiting (p=0.714), pruritus (p=N/A), respiratory depression (p=N/A) levels.

Conclusion: The results of this study demonstrate that use of serratus anterior plane block and thoracic epidural block administration has similar outcomes for post-thoracotomy analgesia.

Keywords: Thoracotomy, bupivacaine, analgesia, pain management, epidural anesthesia

Öz

Amaç: Post-torakotomi ağrısı için günümüzde rejyonal anestezi yöntemleri sıklıkla kullanılmaktadır. Bu çalışmada torakotomi sonrası ağrı için serratus anterior alan bloğunun (SAPB) ve torakal epidural bloğun etkinliğini karşılaştırmayı amaçladık.

Yöntemler: Bu retrospektif çalışmada torakotomi yapılan 120 hasta çalışmaya alındı.Dahil etme kriterlerini karşılayan 70 hasta Grup E (torasik epidural blok) (n=37) ve Grup S (serratus anterior alan blok) (n=33) olmak üzere iki grupta değerlendirildi. Postoperatif 2., 6., 12. ve 24. saatlerde görsel analog skala skorları ve 24 saatlik toplam analjezik tüketimi değerlendirildi. İkincil sonuçlar yan etkiler, ek analjezik ilaç gereksinimi ve komplikasyon olarak belirlendi.

Bulgular: Gruplar arasında yapılan karşılaştırmalarda ameliyat sonrası 2. saat (p=0.417), 6. saat (p=0.271), 12. saat (p=0.734) ve 24. saat (p=0.157) görsel analog skala skorları ve 24 saatlik toplam analjezik tüketimi miktarları (p=0.714) karşılaştırıldığında, 2 grup arasında istatistiksel olarak anlamlı fark yoktu. İki grup arasında yan etkiler, bulantı ve kusma (p=0.714), kaşıntı (p=N/A), solunum depresyonu (p=N/A) düzeyleri açısından istatistiksel olarak anlamlı fark yoktu.

Sonuç: Bu çalışmanın sonuçları, serratus anterior alan bloğu ve torasik epidural blok uygulamasının torakotomi sonrası analjezi için benzer sonuçlara sahip olduğunu göstermektedir.

Anahtar kelimeler: Torakotomi, bupivakain, analjezi, ağrı yönetimi, epidural anestezi

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Ethics Committee Approval: The study wass approved by the local ethical authority.
Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır.

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Introduction

Currently, multimodal approaches are used for post-operative analgesia. Regional anesthesia methods are frequently combined with nonsteroidal anti-inflammatory drugs (NSAID) and opioids as analgesia after major surgery. Pain after thoracotomy can cause ventilation perfusion disorder and hypoxemia together with changes in the lung mechanics [1, 2]. Early causes of the pain were determined as soft tissue damage, incision line, drain entry sites and rib fractures [3]. Multimodal analgesia is required to block sensory signals from all areas due to the large number of pain-producing areas [3]. For thoracic surgery, thoracic epidural block (TEB), paravertebral block and intercostal block are the methods that commonly used [3]. They can be used alone or as a part of a multimodal analgesia. The frequent use of ultrasound (US) in regional anesthesia allows the application of different area blocks. Some of these are the quadratus lumborum block (QLB), pectoral nerves block (PECS), transversus abdominis plane (TAP) block and serratus anterior plane block, which can also be used in surgeries related to abdominal and thoracic wall [4-6].

Serratus anterior plane block (SAPB), which can provide analgesia between the second thoracic vertebra (T2) and the ninth thoracic vertebra (T9) levels, is one of the new plane blocks that can be applied with the coexistence of US that may be a suitable approach for thoracic surgery analgesia [7]. It has been reported that application of local anesthetic drugs to the area between serratus anterior muscle and intercostal muscle can block the cutaneous branches of intercostal muscles in T2-T9 dermatomes [7]. Apart from the thoracic surgery, it has been found that approximately 12 hours of sensory block can be obtained using SAPB which can be used for post-operative analgesia related with the thoracic wall [8].

In this study, we aimed to detect the effects of SAPB and TEB for posterolateral thoracotomy analgesia with regard to the amount of analgesic consumption and visual analogue scale (VAS) scores.

Material and methods

Patients

In this retrospective study, files of the patients who had undergone thoracotomy operation between 2016 and 2018 were reviewed after approval of the local ethics committee (2011-KAEK-25 2018/6-24). The study was performed in accordance with the Declaration of Helsinki. Written consent could not be taken due to the retrospective design of the study.

A total of 120 patients that undergone thoracotomy operation between 18 and 65 years and with American Society of Anesthesiologist (ASA) I-III risk class were included in the study. Exclusion criteria were determined as opioid use before the operation, the second operation history which can change VAS scores, emergency operation and complications which would prolong duration of the surgery and require additional interventions.

27 patients had the lack of the data, ten patients had opioid use before the operation, four patients underwent recurrent surgery, five patients had prolonged surgery due to anatomic difficulties or caused by hemorrhage and four patients were excluded from the study due to emergency surgery.

After exclusion of the patients with these criteria, a total of 70 patients with SAPB (Group S, n= 33) and TEB (Group E, n= 37) were included in the study.

Management of anesthesia

All patients were monitored for non-invasive blood pressure, electrocardiography, heart rate and peripheral oxygen saturation. For induction, 2-2.5 mg /kg intravenous propofol (Propofol 2% Fresenius®, Fresenius Kabi, Bad Homborg, Germany) and 0.6-0.8 mg/kg IV rocuronium bromide (Curon®, Mustafa Nevzat, Istanbul, Turkey) were used. The patients were intubated with double-lumen tube at the appropriate size (35-37 French) and mechanical ventilation was applied to keep end-tidal carbon dioxide as 30-35 mmHg.

For maintenance of general anesthesia, sevoflurane (Sevorane®Likit %100, AbbVie, Queenborough Kent, England) at a concentration of 2-2.5% was given within oxygen-air mixture at a rate of 3 L/min. Additional analgesic requirement was sustained with 1mcg/kg fentanyl (Talinat®, Vem, Istanbul, Turkey).

Treatment of pain

Group E (TEB)

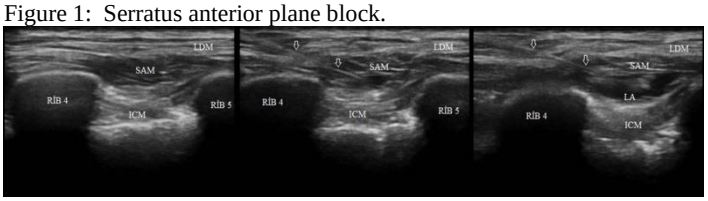
The epidural area was reached in the midline in the 6th (T6) and 7th (T7) thoracic vertebrae with an 18 gauge (G) epidural needle (Braun, Melsungen, Germany) by the method of loss of resistance after appropriate site cleaning before the operation. A 20 G epidural catheter was pushed through the needle after placing the tip of the needle in the epidural space. The needle was then pulled over the catheter and the catheter was placed 3 cm in the epidural space.

After 2 ml of 2% lidocaine (Aritmal®,Osel, Istanbul, Turkey) was administered as a test dose, the patient underwent general anesthesia. At the end of the operation, 15 mL of 0.25 % bupivacain (Marcain® %0,5, AstraZeneca PLC, England) bolus dose was applied after the skin incision was closed.

Group S (SAPB)

SAPB was applied in the lateral decubitus position at the end of the operation when skin incision was closed. After appropriate site cleaning, the first and the second ribs were identified at the midclavicular line with the linear ultrasonic probe. 4th and 5th ribs were displayed moving US probe forward in a sagittal plan towards the caudal direction and; then, serratus, latissimus dorsi and intercostal muscles were traced at the midaxillary line moving posteriorly [7]. After the verification of the area that was provided with a 20 G 100 mm US-visible peripheral nerve block needle (Quincke SonoPlex Pajunk, Geisingen, Germany) by hydrodissection with 3 ml to the inferior plane of the serratus muscle using the in-line technique with the aid of US, 20 ml bupivacaine (Marcain® %0,5, AstraZeneca PLC, İngiltere) injection was applied at 0.25% concentration and a 22 G catheter was placed to 3-4 cm under the serratus anterior muscle (Figure 1). All blocks (TEB, SAPB) were performed by a specialist anesthesiologist who was experienced in the same regional anesthesia after surgery.

Figure 1: Serratus anterior plane block.



SAM: The serratus anterior muscle, LDM: The latissimus dorsi muscle, RIB 4: 4th rib, RIB 5:5th rib, ICM: The intercostalis muscle, LA: local anesthetic, Arrows: Needle

Patient controlled analgesia (PCA)

Both groups through the catheter placed in the epidural space and under the serratus muscle were infused with 100 mg bupivacaine (Marcain® %0,5, AstraZeneca PLC, England) , as 1 mg/mL bupivacaine solution in 100 mL normal saline. PCA settings were a 5 ml intermediate bolus dose and one hour locking time. Tenoxicam (Oksamen-L®, Mustafa Nevzat, Istanbul, Turkey) 20 mg was administered as an analgesic drug to all patients before the post-operative extubation. When Visual

Analogue Scale (VAS)> 5, for additional analgesic requirement was ordered to be given.

As primary measures, VAS scores (2nd, 6th, 12th and 24th hours after operation) and total analgesic consumption for 24 hours were analyzed. Secondary measures were side effects (nausea and vomiting, pruritus, respiratory depression, bradycardia, hypotension), additional analgesic requirement (Tramadol, Paracetamol, Pethidine HCl), intraoperative opioid requirement. The patients whose mean arterial pressures were below 60 mmHg postoperatively were considered as hypotensive and recorded. Bradycardia was accepted as heart beats below 50 beats/min and respiratory depression has been identified as the mask was considered to be O2 (4lt/min) and SpO2 below 95.

Statistical analysis

Analysis of the data was done using the IBM SPSS 22.0 statistical package program. The descriptive statistics were given as mean± standard deviation, with median (minimum and maximum) for quantitative variables, and as percentages for categorical variables.

In the evaluation of the study data, chi-square (χ^2) test was used for comparison of qualitative data as well as descriptive statistical methods. The normal distribution of the data was evaluated by the Shapiro-Wilk test. Mann-Whitney U test was used in the comparison between the groups. Probability values smaller than (P) α= 0.05 were accepted as significant.

Results

In the study population, 57 were males and 13 were females. The mean age was 53.35 ± 7.19 years. The mean body mass index (BMI) was 23.28 ± 1.84 kg/m2. Surgical side was the right and the left in 38 and 32 patients, respectively. The mean duration of the surgery was 134.2±26.8 min.

Postoperative 2nd, 6th, 12th, 24th hour visual analogue scale scores were measured as 3.2±0.71, 2.8±0.87, 1.80±0.86 and1.94±0.83 in Group S, respectively. These scores were measured as 3.05±0.68, 2.65±0.63, 1.88±0.93 and 1.65±0.63 in Group E, respectively.

Between the groups; there was no statistically significant difference in terms of gender, age, BMI, surgical side and duration of the operation (p>0.05 for all) (Table 1). In VAS scores and the amounts of post-operative analgesic consumption including tramadol (p=0.572), NSAID (p=0.079) and opioid drugs (p=0.558), no statistically significant difference was found between the groups (Table 2, 3). When the total local anesthetic consumption with PCA during the postoperative 24 hours were examined, it was 23.28±5.8 mg 27±11.6 mg in Group S and Group E, respectively. There was no significant difference between the groups (p=0.714) (Figure 2). There was also no statistically significant difference between the groups in terms of the type of the side effects (p> 0.05 for all) (Table3).

	Group S (n=33)	Group E (n=37)	p
Age (year)[¥]	54±7.4	52.6±6.9	0.429
BMI (kg/m^2)[¥]	23.2±1.8	23.3±1.84	0.986
Gender[&] (M/F)	28/5	29/8	0.490
Amount of opioid given during operation (µg) [¥]	124.12 (80-200)	114 (80-180)	0.289
Surgical side (right/left)	17/16	21/16	0.663
Duration of the surgery (min) [¥]	137.2±24.8	133.3±23	0.358

Table 1. Comparison of the demographic characteristics of the patients.

BMI: body mass index, M: male, F: female, [¥] mean ±SD, [&]: median (min-max) value

Figure 2: Total amount local anesthetics during 24 hours.

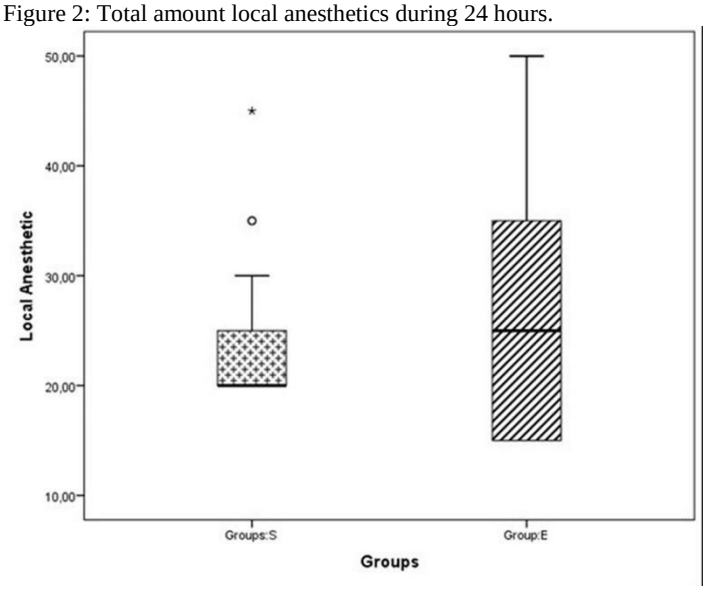


Table 2: Comparison of VAS scores at rest between groups

VAS	Group S (n=33)	Group E (n=37)	p
2ndhour [¥]	3.2±0.71	3.05±0.68	0.417
6thhour [¥]	2.8±0.87	2.65±0.63	0.271
12thhour [¥]	1.80±0.86	1.88±0.93	0.734
24thhour [¥]	1.94±0.83	1.65±0.63	0.157

VAS: Visual Analogue Scale, [¥]: mean ±SD

Table 3: Side effects and additional analgesic requirement.

Variable		Group S (n=33)	Group E (n=37)	p
Side effects	Nausea and vomiting[µ]	-	1(3.4%)	0.309
	Pruritus	-	-	N/A
	Respiratory depression	-	-	N/A
	Bradycardia	-	-	N/A
	Hypotension	-	-	N/A
Analgesic requirement	Tramadol (mg)[¥]	75±52.1	82.8±44	0.572
	Paracetamol (gr)[µ]	1 (2.9%)	3 (8.6%)	0.079
	Pethidine HCl (mg)[µ]	50 (2.9%)	100 (5.7%)	0.558

[µ]: frequency (%), [¥]: mean ±SD

Discussion

We retrospectively reviewed the patients who underwent SAPB and TEB in order to provide analgesia after thoracotomy operation. There was no statistically significant difference between two groups considering the amount of analgesic consumption and VAS scores.

Recently, pain after many surgical procedures has been treated with multimodal analgesia approach. These methods combine intravenous drug administration with regional anesthesia procedures. These methods reduce the side effects of opioids in analgesic doses and improve patient comfort [9]. Multimodal approaches for thoracic surgery can be applied in combination with intravenous opioid, NSAIDs and regional methods such as paravertebral block and thoracic epidural block.

Although thoracic epidural block is accepted as the gold standard treatment for thoracic surgery, side effects such as neuroaxial hematoma, urinary retention and hypotension are the faced difficulties [10, 11]. The complications such as total spinal block and neural damage observed following paravertebral block application which can be used as an alternative have been mentioned in the literature as limitations of this method [12].

Current regional anesthesia methods have focused on fascial blocks with the widespread use of US. Effectiveness of SAPB, which is used more frequently for thoracic anatomy, have been tried to be determined with case studies and research articles in the literature. Breast surgery, thoracotomy and video assisted thoracoscopic surgery operations are some of the studies using SAPB [9, 13-15]. Although the level of analgesia is indicated as level of T2-T9 by Blanco et al. [7], there are also publications in which the sensory block level is stated to be formed in a narrower area of T2-T6 to T3-T8 [8,15-20]. In a randomized controlled study of SAPB for postoperative pain after radical mastectomy, when compared with the other group in which the paravertebral block was administered, although they have reached low values of VAS and morphine consumption in the SAPB group, they reported that the effect was limited [8]. In another study investigating SAPB efficacy, Ökmen et al. [21] used patients who underwent VATS. In this study using bupivacaine in 20 mL of 0.25 % concentration, they found a lower amount of tramadol consumption postoperatively and found that this block was effective after VATS [14]. In one randomized controlled study by Khalil et al. [11], SAPB and TEB applications were compared in two groups with 20 patients. They reached similar VAS scores and opioid consumption values after local anesthetic infusion via catheter after single-dose medication. They shared that SAPB block application maintains better hemodynamic stability [11]. Another study retrospectively tried to determine efficacy of SAPB administration and reported that it was an alternative method for thoracotomy analgesia [15].

The presence of few studies of post-thoracotomy pain limits the discussion of efficacy and complications. There are different uses of amounts of local anesthetics for SAPB in the literature [11, 15, 16, 20, 21]. Although 20 mL of local anesthetic in our study showed sufficient analgesia for 24-hour follow-up for thoracotomy analgesia, Khalil et al. [11] used higher doses of local anesthetics (30 ml bolus and 5 ml/h infusion) for similar analgesia level in their study. Although the dosage of local anesthetic for optimal analgesia can be determined in future studies, as in other plane blocks, the use of high amounts of local anesthetics seems to be remarkable. On the other hand, we think that the use of greater amounts of local anesthetics in the epidural block group may be important even though there is no statistically significant difference between groups in terms of the 24 hours total local anesthetic amounts. With these results, single-dose SAPB administration appears to be an adequate supportive treatment option for post-thoracotomy pain.

Retrospective design and absence of follow up after sensory block were the limitations of this study.

In conclusion, the results of this study suggest that SAPB provides analgesia at a level similar to that of TEB and is effective as a supportive treatment option. The higher local anesthetic dose with in single shot SAPB administration can be seen as a disadvantage of the block.

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Utility of Nestin immunohistochemistry in the diagnosis of granular cell tumor

Granüler hücreli tümör tanısında Nestin immunhistokimyasının kullanımı

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Abstract

Aim: Granular cell tumors (GCTs) show neuroectodermal differentiation. Morphologically, a wide variety of mesencymal tumors may have granular cell appearance. Nestin, which is an intermediate filament protein expressed in undifferentiated cells during central nerves system development and its tumors. We aim to determine the diagnostic utility of Nestin in diagnosis of GCTs.

Methods: Nestin immunohistochemistry applied to GCT cases and other mesenchymal tumors which may have granular cytoplasm and the major differential diagnostic consideration of GCT. A total of 21 GCT from different tissues (including 7 in the esophagus, 8 originating from skin tissues, 4 in the tongue, 2 in the vocal cord), 17 gastrointestinal stromal tumor in the gastrointestinal tract, 8 leiomyoma (5 in the esophagus and 3 originating from skin tissues), 4 schwannoma (1 in the esophagus and 3 originating from skin tissues), subcutaneous mesenchymal tumors (including 7 neurofibroma, 5 fibroma, 15 dermatofibroma), 20 melanocytic nevi, 15 gastric xanthomas and 15 xanthalesma of the skin were included the study.

Results: Nestin positivity was detected in all GCTs. Additionally, strong Nestin positivity was seen in all gastrointestinal stromal tumors, schwannoma and neurofibroma cases. However, Nestin was negative in all leiomyoma, fibroma, dermatofibroma, melanocytic nevi, gastric xanthoma and xanthalesma cases.

Conclusion: The study showed that Nestin immunohistochemistry has limitation in distinction of GCT from tumors arising from neural cell lineage such as gastrointestinal stromal tumor, schwannoma and neurofibroma; however, Nestin as a neural marker provides an evidence to neural origin of GCT and could be useful in distinction GCT from other mesenchymal tumors with granular cytoplasm such as leiomyoma, dermatofibroma and melanocytic nevi.

Key words: Nestin, granular cell tumor, neural origin

Öz

Amaç: Granüler hücreli tümorler (GCTs) nöroektodermal diferansiasyon gösterirler. Morfolojik olarak pek çok mezenkimal tümor, granüler hücreli görünüme sahip olabilir. Nestin, santral sinir sistemi gelişimi boyunca undiferansiye hücrelerde ve santral sinir sistemi tümörlerinde eksprese edilen bir intermediate filament proteindir. Çalışmamızda Nestin proteininin granüler hücreli tümör ayırıcı tanısında kullanılabilirliğinin belirlenmesi amaçlanmıştır.

Yöntemler: Çalışmamızda GCT tümör olguları ve granüler sitoplazmaya sahip olabilecek ve granüler hücreli tümör ayırıcı tanısında başlıca ele alınan mezenkimal tümörlere immunhistokimyasal olarak Nestin antikoru uygulanmıştır. Farklı dokulardan köken alan 21 GCT (7'si özofagus, 8'i deri, 4'ü dil, 2'si vokal korddan gelişen) , 17 GIST (gastrointestinal stromal tumor- gastrointestinal kanaldan gelişen) , 8 leiomyoma (5'i özofagus, 3'ü deriden gelişen), 4 schwannoma (1'i özofagus, 3'ü deri yerleşimli) ve subkutan yerleşimli mesenkimal tümörler (7 nörofibroma, 5 fibroma, 15 dermatofibroma) ile 20 melanositik nevüs, 15 gastrik ksantom ve 15 ksantalezma olgusu çalışmamıza dahil edilmiştir.

Bulgular: GCT'lerin tamamında Nestin pozitifliği görüldü. Ayrıca tüm GIST'ler, schwannoma ve nörofibromada Nestin pozitif olarak izlendi. Bununla birlikte tüm leiomyoma, fibroma, dermatofibroma, melanositik nevüs, gastrik ksantom ve ksantalezma olgularında Nestin ekspresyonu görülmedi.

Sonuç: Çalışmamız Nestin immunhistokimyasının GCT'ün ayırıcı tanısında özellikle GIST, Schwannoma ve nörofibroma gibi nöral orijinli tümörlerde sınırlı olduğunu göstermektedir. Bununla birlikte nöral bir marker olan Nestin GCT'ün nöral kökenine dair bir kanıt sunmaktadır. Nestin, GCT 'ün granüler sitoplazmaya sahip leiomyoma, dermatofibroma gibi diğer mezenkimal tümörler ve melanositik nevüsden ayırımında kullanılabilir.

Anahtar kelimeler: Nestin, granular hücreli tümör, nöral orijin

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Introduction

Granular cell tumor (GCT) is a rare and usually benign tumor having neuroectodermal differantiation. About 2 % of the cases can present a malignant course [1]. While GCTs have been reported in various parts of the body such as the skin and the viscera, they most frequently occur in the head and the neck regions. Around 40 % of the cases are found in the tongue while the skin and subcutaneous tissue share one third of the cases. Other sites such as the esophagus, the stomach, the larynx, the bronchus, the uvea, soft tissue and the pituitary stalk may also be involved. GCTs are usually poorly circumscribed and non-encapsulated. However, encapsulated GCTs can occur, especially the skin and the subcutaneous tissue.

Histologic features of GCT are quite distinctive with abundant granular-appearing of tumor cells. However, granular cell changes due to lysosome accumulation can be observed in a variety of neoplasms and they might closely resemble one another [2]. Therefore, a wide range of tumor and reactive pathologies such as xanthoma and trauma associated changes should be consider in differential diagnosis of GCTs.

Currently, the hypothesis of the neural origin of GCT is widely accepted. Immunohistochemical studies also showed Schwann cell origin of GCT through the positive identification of S-100 protein [3-5]. Recently, Nestin expression has been shown in GCT of gastrointestinal (GI) tract [6, 7]. Nestin protein was firstly discovered in the developing nervous system of mice. Nestin is considered to be a marker of neural stem and skeletal muscle progenitor cells and tumors related to such cell lineages, although its expression in other cell types is under investigation [8]. It has been detected in many kinds of tumors, especially in tumors derived from the central nerves system such as gliomas, medulloepitheliomas, primitive neuroectodermal tumors, meningiomas [9].

The purpose of this study was to investigate the diagnostic benefit of Nestin immunohistochemistry as a neural tissue marker in the diagnosis of GCTs.

Material and methods

Patients and clinical data

This retrospective study consisted of 21 cases of GCT diagnosed at Baskent University, Faculty of Medicine, Department of Pathology between 2003 and 2017. Written consent and ethical approval could not be taken due to the retrospective design of the study.

The diagnosis of GCT cases have been detected from the archives of pathology report collected via electronic files on computer. Demographic data including age, gender and sites was noted by using the patient's medical records from the archives of the pathology, retrospectively.

The location of 21 GCT cases were from diverse anatomical sites. We categorized the patients into three groups according to their locations (the GI tract, the skin and the head and neck lesions). Seven cases of GCT were from gastrointestinal tract (the esophagus), eight cases of GCT were from the skin (three on the extremities, one on the vulva, one on the breast, one on the abdominal surface, two on the face) and six cases of GCT were from the head and neck region (four on the tongue, two on the vocal cord).

To investigate the specificity of Nestin, a total number of 76 cases of other mesenchymal tumors which are considered mostly in the differential diagnosis of GCTs and 15 cases of gastric xanthomas and 15 cases of xanthalesma from the skin were also applied Nestin immunohistochemistry.

Histologic types of tumor which are considered mostly in the differential diagnosis of GCTs was determined according to literature [2]. This mesenchymal tumor group were also consisted of tumors which are located similar site of GCTs. Pathology reports were reviewed and mesenchymal tumor group were determined from archives of the pathology department between and 2003 and 2017. In the mesenchymal tumor group, tumor affected the GI tract includes five leiomyoma of the esophagus, one schwannoma of the stomach and 17 gastrointestinal stromal tumor (GIST) cases. Tumors affected the skin and the soft tissue includes three leiomyoma, three schwannoma, seven neurofibroma, five fibroma, 15 dermatofibroma and 20 melanocytic nevi. Gastric xanthomas (n=15) and xanthalesma (n=15) were also studied. Among them, two leiomyoma of the esophagus, one leiomyoma of the skin, one melanocytic nevi, all gastric xanthomas and xanthelesma of the skin have granular cell appearance.

Demographic and clinicopathogic features of patients are given in table I.

Pathological analysis

All H&E-stained slides were reviewed by a single pathologist and the diagnosis was confirmed. Immunohistochemical staining for S-100 protein, CD68 had been used for the confirmation of the diagnosis of GCT before. Representative samples with GCT were selected for immunohistochemical analysis with Nestin. To investigate the specificity of Nestin, other tumors which are considered in the differential diagnosis of GCTs were also applied Nestin immunohistochemistry.

Immunohistochemical staining

Formalin-fixed paraffin-embedded tissue was used for the immunohistochemical reactions. Three-µm thick sections were obtained from the formalin-fixed, paraffin-embedded tissue blocks mounted on positively charged glass slides, and dried overnight at room temperature. Sections were de-paraf¬finized in xylene and in graded ethanol and placed in 0.5% hydrogen peroxide in methanol for 5 minutes to block endogeneous peroxidase activity. Antigen retrieval was carried out by incubation in 0.01 M citrate buffer (pH 6.0) for 15 minutes in a microwave oven. Sections were removed and put in phosphate-buffered saline for 15 minutes, following rinsed thoroughly in deionized water. The sections were exposed to the primary antibody for 60 minutes at room temperature. The standard streptavidin-biotin-peroxidase complex method was used for Nestin antibody, 1:100 dilution; (Santa Cruz-sc-71665), by employing di-amino-benzidine as the chromogen.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and median value if necessary. Categorical variables were expressed as frequencies with percentages.

Results

The histological features were similar in all GCTs. At low magnification, tumors were composed of sheets or nests of plump, round or slightly ovoid cells with abundant eosinophilic granular cytoplasm with small, round, centrally located uniform pyknotic nuclei (Figure 1). Histochemically, tumor cells were characterized by globular and diffuse periodic acid-Schiff positivity of the cytoplasm, which remained after diastase digestion. The following primary antibodies were used to confirm diagnosis: S100 and CD68. All of the cases of GCTs were positive for S-100 and CD-68. Nestin positivity was also

detected in the cytoplasm of tumor cell with diffuse and strong staining intensity (Figure 2).

In mesenchymal tumor group, strong Nestin positivity was seen in all GIST, schwannoma and neurofibroma. Nestin was negative in all leiomyoma, fibroma, dermatofibroma, melanocytic nevi, gastric xanthoma and xanthalesma. Nestin was also positive at the endothelium of vessels in both study group. The results of the immunohistochemical studies on these tumors are summarized in Table 2.

Pathology*	Anatomic site	Location*	Sex F/M	Age (year)	Size (mm)
Granular cell tumor (21)	Gastrointestinal	Esophagus (7)	4/3	42.7	7.71
	Skin	Leg (3), vulva (1), breast (1), abdominal (1), face (2)	4/4	46.3	11.2
	Head&neck	Tongue (4)	5/1	54	11
		Vocal cord (2)		46	4.5
Leiomyoma (8)	Gastrointestinal	Esophagus (5)	3/2	54	11.0
	Skin	Leg (3)	3/0	62	13
Schwannoma (4)	Gastrointestinal	Stomach (1)	1/0	18	9
	Skin	Arm (2), face (1)	1/2	39	10
GIST (17)	Gastrointestinal	Stomach (9), duodenum (5), liver (3)	9/8	55.9	73
Neurofibroma (7)	Skin	Extremity (7)	4/3	46.3	12
Fibroma (5)	Skin	Scalp (2), extremity (2), lip (1)	3/2	54.8	13
Melanocytic nevi (20)	Skin			49.4	7
Dermatofibroma (15)	Skin	Hand (1), trunk (5), extremity (9)	11/4	47.3	9
Xanthoma (15)	Gastrointestinal	Stomach (15)	8/7	51	5
Xantholesma (15)	Skin	Eyelid (10), face (3), extremity (2)	9/6	55	6

Table 1. Demographic and clinical features of the patients.

*: (n), F: Female, M: Male, GIST: Gastrointestinal stromal tumor

Discussion

In the current study, we found that Nestin was uniformly expressed in GCTs which were obtained from various tissues. Similar to us, Parfitt et al. [6] demonstrated the presence of Nestin expression in GCT before. Their results were related to the GCT originated only from the GI tract. In the present study, the majority of the GCT was from the GI tract, the cutaneous tissue and less commonly from the head and the neck regions. In the GI tract, GCTs are preferentially found in the esophagus, followed by the colon and the rectum and the perianal region [7]. Similar to the literature, we found that majority of the GCT cases were localized in the esophagus in the GI tract.

Histologically, all GCTs were composed of well-demarcated proliferations of pale-stained polygonal cells with the characteristic eosinophilic granular cytoplasm and an oval nucleus. The granules stain positive with PAS but are resistant to diastase. The diagnosis was verified using immunohistochemical staining with S100 and CD68. S100 is the most widely used antibody which supports the neural nature of the GCTs. Another antibody is CD-68 which is usually positive in the GCTs. CD68 positivity is attributed to an intracytoplasmic accumulation of phagolysosomes and does not reflect the histiocytic origin of the tumor [5]. In a wide variety of both benign and malignant tumors, they may show focally or extensively granular cell change [2]. Granular cell differentiation is due to an increased number of secondary lysosomes and hallmarked by the presence of abundant eosinophilic granularity of cytoplasm. In the differential diagnosis of GCT, we consider leiomyoma [10], schwannoma [11, 12], GIST [6] and xanthoma in GI tract, leiomyoma [13, 14], neurofibroma, dermatofibroma, melanocytic nevi [15] and xanthalesma in the skin lesions and fibroma in the oral lesions.

Table 2. Immunhistochemical Nestin expression in GCT and other tumors.

Tumor*	Nestin Positive*	Negative*	Location
GCT (21)	21	--	GI tract, cutaneous , head and neck
GIST (17)	17	--	GI tract
Schwannoma (4)	4	--	GI tract, cutaneous
Leiomyoma (8)	--	8	GI tract, cutaneous
Neurofibroma (7)	7	--	Cutaneous
Fibroma (5)	--	5	Cutaneous
Melanocytic nevi (20)	--	20	Cutaneous
Dermatofibroma (15)	--	15	Cutaneous
Xanthoma (15)	--	15	GI tract, cutaneous
Xanthalesma (15)	--	15	Cutaneous

*: (n)

Figure 1. Tumors showing sheets or nests of plump, round or slightly ovoid cells with abundant eosinophilic granular cytoplasm with small, round, centrally located uniform pyknotic nuclei.

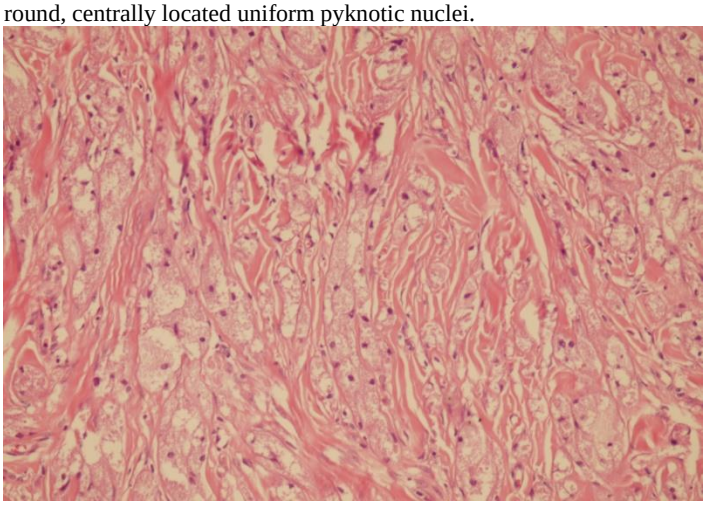
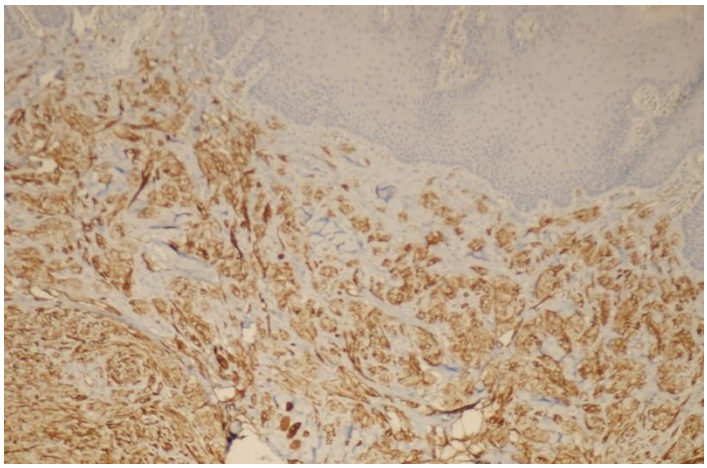


Figure 2. Nestin positivity in the cytoplasm of tumor cell with diffuse and strong staining intensity.



In our study, Nestin was positive in GISTs, schwannomas and neurofibromas. Similar to us, Sarlomo et al. [11] have shown Nestin expression in GIST and GI Schwannomas. In literature there are conflicting results on Nestin expression in schwannoma. In the present study, we demonstrated Nestin positivity in both GI and skin schwannoma (1 the esophagus and 3 the skin). On the other hand, Tsujima et al. [16] have reported no evidence of Nestin expression in schwannoma which developed in the GI tract but they showed strong immunreactivity for Nestin in all their GIST cases. However, their study includes only one case of Schwannoma in the GI tract. A study of large series of cases presented by Hou et al. [17] have showed that Nestin expression was variably positive in thirty-three cases of GI schwannomas (78.8%, 26/33) . In another study, Shimada et al. [18] reported weak Nestin expression in 10/10 schwannomas from soft tissue. On the basis of these results, we thought that further studies including more patients need to clarify the Nestin expression in schwannomas. However, our results indicated that Nestin expression could not be used to distinguish GCT from GIST, Schwannomas and neurofibroma.

In addition, our results showed that Nestin may be helpful in separating GCT from leiomyoma. We found that Nestin was uniformly negative in all leiomyomas. Nestin is known to be expressed in skeletal muscle progenitor cells, but is down-regulated on cellular differentiation [19-21]. Similar to us, studies showed that Nestin expression has not been identified in GI leiomyomas [11, 16] and leiomyomas arises soft tissue [8]. Actually, GCTs are can be distinguished from leiomyomas by immunhistochemical staining with smooth muscle actin (SMA) and desmin. While SMA and desmin reactions confirm the smooth muscle origin of the cells, negative staining with Nestin also suggests that they are unlikely to be of neural origin in leiomyomas. On the other hand, in contrast to benign muscle tumors (i.e. leiomyomas), authors have found that malignant tumors with muscle differentiation, rhabdomyosarcoma and leiomyosarcoma, showed strong Nestin expression in the majority of cases [18]. Authors have thought that Nestin expression may correlate with malignant transformation of the myogenic tumors.

Beside leiomyoma and schwannoma, we have evaluated Nestin expression in other skin lesions such as dermatofibroma, melanocytic nevi and xanthalesma. Although most of them are benign but diagnostic difficulty may arise for lesions with xanthomatous cells. In the study, none of the cases of dermatofibroma, melanocytic nevi and xanthalesma showed Nestin expression. Therefore we thought that Nestin can help to differentiate GCT from the melanocytic nevi and dermatofibroma. Similar to us authors found that the melanocytic nevi, even dysplastic nevi, showed no or weak staining with Nestin. However increased Nestin expression has been shown to be associated with aggressive melanoma features [22, 23]. Similarly, while diffuse positive Nestin expression in dermatofibrosarcoma protuberans cases, a partial positive reaction was reported in dermatofibroma cases [24].

GCT is mostly benign, although in 1% to 3% of cases, a malignant development can occur. The histologic features such as high mitotic index and a cellular/nuclear pleomorphism are related to malignancy. Initially, because of non-neoplastic nature of the GCT, it has been considered, as a degenerative process that resulting from trauma or as a storage disorder involving histiocytes, in the etiology. In the present study Nestin expressson was not detected in histiocytes rich lesion such as xanthomas, xanthalesmas, dermatofibromas but GCTs. Therefore, we thought that Nestin can be useful in distinguishing the GCT from the histiyocytic lesions and Nestin positivity provide an evidence for neural origin of the GCTs other than histiocytic origin.

To confirm the neural origin of GCT, authors have investigated several molecules such as p75, Calretinin, Protein gene product 9.5 (PGP 9.5), Inhibin-alpha, Galectin-3, HBME-1 and Sox10 in GTCs [25-32]. Among those antibodies, Inhibin-alpha, Galectin-3 and HMBE are not only isolated from the neural tissues but their expression has been reported in the neural tumors, such as schwannoma, neurofibroma, ganglioneuroma and malignant peripheral nerve sheath tumor, before [25-27]. On the other hand P75, Calretinin, PGP 9.5 and SOX10 have been originally isolated from neural tissues and thought to be specific for neural and nerve sheath differentiation [28-32]. However, these proteins are also expressed in many non- neural and non-nerve sheath tumors [23]. Nestin seems to be more specific for supporting the neural origin of the GCTs but its expression in other cell types is under investigation [3]. We also demonstrated that Nestin expression was seen in neuronal lineage tumors such as GISTs, schwannoma, neurofibroma.

Due to the rarity of GCT, our study has limited cases. Another limitation of our study is that our case series includes only benign tumors because of the GCTs are rarely malignant. Therefore, we also consider only benign tumors in differential diagnosis of GCT.

However the present study demonstrated that GCTs which are originated from varies tissue showed strong and diffuse positive Nestin expression.

In conclusion, based on the results of the present study, Nestin could be considered as a useful immunohistochemical marker for identifying the GCTs. Further studies may plan to determine the role of Nestin in differential diagnosis of malignant GCT.

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Prevalence of dermatoses among pediatric patients in a secondary hospital in Eskisehir, Turkey: A cross-sectional study

Eskişehir'deki ikinci basamak sağlık merkezine başvuran çocuk hastaların deri hastalıkları prevalansı: Bir kesitsel çalışma

Hamza Yıldız [1]

Abstract

Aim: To determine prevalence of skin diseases among pediatric patients in Eskisehir, Turkey.
Methods: This was a cross-sectional study. Medical record of the outpatient clinics of Dermatology in Eskisehir Yunus Emre State Hospital was retrospectively assessed. Children (between 0 and 18 years old) who attended the dermatology out-patient clinic between January 2017 and December 2017 were included in the study.
Results: A total of 9,057 patients were included in the study and 4,204 (46.41%) patients were male. The ten most frequent diagnoses and their prevalences were: acne (27. 7%), viral warts (13.6%), contact dermatitis (13.2%), xerosis cutis (8.1%), seborrhoeic dermatitis (6.4%), urticaria (6.2%), bacterial infections (4.3%), dermatophytosis (3.7%), nevus (2.5%), and atopic dermatitis (2.5%).
Conclusions: Acne, viral warts, and contact dermatitis were the three most common skin diseases that we detected in pediatric age groups Eskisehir, Turkey. In order to reveal the prevalence of pediatric skin diseases, further epidemiological studies are needed.
Key Words: Epidemiology, pediatric dermatology, skin diseases

Öz

Amaç: Eskişehir'deki pediatrik hastaların deri hastalıkları prevalansını ortaya koymak.
Yöntemler: Bu araştırma kesitsel bir çalışmadır. Eskişehir Devlet Hastanesi Dermatoloji Polikliniği'ne başvuran hastaların kayıtları otomasyon dosya sisteminden retrospektif olarak analiz edildi. Ocak 2017 ile Aralık 2017 tarihleri arasında dermatoloji polikliniğine başvuran çocuk hastalar (0-18 yaş arasındaki) çalışmaya dahil edildi.
Bulgular: Çalışmamıza 4.204 (%46,41)'ü erkek, toplam 9.057 hasta dahil edildi. En sık rastlanan on hastalık ve prevalansı şu şekildeydi: akne (%27,7), viral siğiller (%13,6), kontakt dermatit (%13,2), kserosis kutis (%8,1), seboreik dermatit (%6,4), ürtiker (%6,2), bakteriyel enfeksiyonlar (%4,3), dermatofitozlar (%3,7), nevüs (%2,5) ve atopic dermatit (%2,5).
Sonuç: Eskişehirde akne, viral siğiller ve kontakt dermatitin en sık karşılaşılan üç pediyatrik dermatolojik hastalık olduğu tespit edildi. Pediatrik deri hastalıkları prevalansını ortaya koymak için daha fazla epidemiyolojik çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Deri hastalıkları, epidemiyoloji, pediatrik dermatoloji

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Introduction

Development of skin diseases is influenced by external factors, such as socioeconomic status, climate, geographic region, personal habits, and also internal factors, such as gender, age, and heredity. The prevalence of skin diseases differs between regions as a result of these factors [1].

Additionally, physiology of the skin tissue in children is very different from adults. Moreover, there are many physiological and anatomical differences between newborn, infants, preschoolers, school-age children, and adolescents. Therefore, the frequency, distribution and characteristics of the diseases seen in children are different from adults. Moreover, the prevalence of skin diseases among age groups in children is different depending on all these factors [2].

The prevalence of skin diseases in pediatric patients is very important in planning preventive and therapeutic healthcare services [1].

This study was performed in Eskisehir. It is located in the Central Anatolia region of Turkey and has an altitude of 732 meter. The average air temperature values in the most recent 40 years measured between - 3.7°C and + 29°C. The city was in the 7th line in the evaluation of national socioeconomic development [1].

Information on the prevalence of skin disorders in pediatric patients is limited. Best of our knowledge; this research article was the first prevalence study in children in Eskisehir, Turkey. We aimed to clarify the prevalence of skin disorders among pediatric patients in Eskisehir.

Material and methods

This study was a cross-sectional study. Ethical approval was taken from the local ethics committee. The study protocol complied with the ethical guidelines of the Declaration of Helsinki of the World Medical Association. Written informed consent could not be obtained from the parents of the patients due to retrospective design of the study.

The medical records of the outpatient clinics of Dermatology in Eskisehir Yunus Emre State Hospital were retrospectively assessed. Children who attended the dermatology outpatient clinics between January 2017 and December 2017 were included in the study. Physical examinations were performed by seven different dermatologists. A total of 30,001 applications were recorded at the dermatology out-patient clinics of Eskisehir Yunus Emre State Hospital. Of the 30,001 patients, 12,872 (42.9%) were children who were under 18 years old. Only one application per patient was included in the study. In order the find accurate prevalence, we only included new patients in the present study. Of the 12,872 patients, 3,815 patients (29.6%) were excluded from the study and 9,057 new patients were included in the study.

The patients were diagnosed based on anamnesis and clinical features and confirmed by laboratory tests (e.g. fungal direct examination) or skin biopsy when indicated. The International Classification of Diseases (ICD-10) was used in order to classify the diagnoses.

The patients were divided into four groups according to the age groups as follows: newborn and infant (0-2 years), preschoolers (3-5 years), scholars (6-11 years) and adolescence (12 – 17 year).

Statistical Analysis

Data were entered into an Excel spreadsheet and analyzed with the Statistical Package for the Social Sciences (SPSS 15.0 Statistical software, SPSS Inc., Chicago, IL, USA). The calculated values are given as the mean values ± standard deviation (SD). Student's t-test was used to compare mean age. The Mann-Whitney U test was used for quantitative data without normal distribution. The Chi-square test was used to compare qualitative data. A p value less than 0.05 was considered to be statistically significant.

Results

Of 9,057 patients, 4,204 (46.4%) were male and 4,853 (53.6%) were female. Male to female ratio was 0.86. Mean age of the patients was 11.7±4.9 years (median age 13, the age range 0-17 years). Mean age of the female children was 11.92±4.67 years (median age: 13, the age range 0-17 years). In the male children, it was 11.48±5.05 years and median age was 13 years (the age range 0-17 years).

There was statistically no significant difference between sex and age (p=0.784).

Of 9,057 patients, 545 (6.0%) were newborn and infant, 636 (7.0%) were preschoolers, 2,778 (%30.7%) were scholars, and 4,953 (%54.7%) were adolescence.

Of 9,057 patients, 1,185 (13.1%) patients were diagnosed with more than one skin disease. A total of 125 skin diseases were recorded.

Firstly, we evaluated the prevalence of skin disease of the patients. The most frequent diagnoses in all age groups and their prevalence were: acne (n=2,506; 27.7%), viral warts (n=1,231; 13.6%), contact dermatitis (n=1,195; 13.2%), xerosis cutis (n=730; 8.1%), seborrhoeic dermatitis (n=579; 6.4%), urticaria (n=560; 6.2%), bacterial infections (n=386; 4.3%), dermatophytosis (n=334; 3.7%), nevus (n=229; 2.5%), atopic dermatitis (n=226; 2.5%). Age distribution of the most common skin diseases is represented in Table 1.

There were statistically significant differences between female and male for acne, seborrhoeic dermatitis, and urticaria ($p<0.05$ for all). Acne, seborrhoeic dermatitis, and urticaria were more common in female pediatric patients than male (Table 2). Males showed a greater susceptibility to warts, bacterial infections, and dermatophytosis, whereas females were more susceptible to contact dermatitis, xerosis cutis, and nevus. But, there was no statistical difference ($p>0.05$ for all).The distribution of the most common ten diseases according to gender is shown in Table 2.

Secondly, we evaluated the prevalence of pediatric skin diseases for each age group.

I) In the newborn and infant group, the most frequent diagnoses were: atopic dermatitis (n=76; 13.9%), xerosis cutis (n=40 ; 7.3%), contact dermatitis (n=21; 3.9%), bacterial infections (n=19 ; 3.5%), urticaria (n=19; 3.5%), seborrhoeic dermatitis (n=16; 2.9%), intertrigo (n=14; 2.6%), nevi (n=10; 1.8%), milaria (n=6; 1.1%), pityriasis alba (n=3; 0.6%).

II) In the preschoolers group, contact dermatitis (n=250; 39.3%) was the most prevalent dermatoses, followed by xerosis cutis (n=161; 25.3%), urticaria (n=95; 14.9%), viral warts (n=88; 13.8%), atopic dermatitis (n=64; 10.1%), bacterial infections (n=62; 9.8%), molluscum contagiosum (n=42; 6.6%), seborrhoeic dermatitis (n=38; 5.9%), pruritus (n=26; 4.1%) and nail dystrophies (n=25; 3.9%).

III) In the scholars group (n=2,778), the most frequent diagnoses were: viral warts (n=620; 27.2%), contact dermatitis (n=460; 20.2%), xerosis cutis (n=304; 13.4%), acne (n=202; 8.9%), urticaria (n=196; 8.6%), seborrhoeic dermatitis (n=195; 8.6%), bacterial infections (n=113; 4.9%), nevi (n= 85; 3.7%), atopic dermatitis (n=63; 2.8%), alopecia areata (n=62; 2.7%).

IV) Among 4,953 cases, in the adolescence group, the top ten skin disorders were, in descending order of incidence, acne (n=2,297, 46.4%), viral warts (n=513 ; 10.4%), contact dermatitis (n=445 ; 8.9%), seborrhoeic dermatitis (n=330; 6.7%), xerosis cutis (n=225; 4.5%), bacterial infections (n=192; 3.9%),

urticaria (n=248; 5.0%), telogen effluvium (n=133; 5.0%), nevus (n=117; 2.4%), hyperhidrosis (n=104; 2.1%).

Thirdly, we have assessed the prevalence of disease groups. The rate and frequencies of the disease groups are represented in Table 3.

The most common skin disease in the age groups is as follows; Atopic dermatitis in the 0-2 age group (13.9%), acne vulgaris in the adolescence group (46.4%), viral warts in scholars group (22.3%) and contact dermatitis in the preschoolers group (39.3%) (Table 1).

Table 1: Age distribution of the most common skin disease.

No	Disease	Age groups: Infant (0-2 year) n = 545		Preschoolers (3-5 year) n = 636		Scholars (6-11 year) n = 2,778		Adolescence (12-17 year) n = 4,953		Total
		n	%	n	%	n	%	n	%	
1	Acne	2	0.4	5	0.8	202	7.3	2,29	46.4	2,506
2	Contact dermatitis	40	7.3	250	39.3	460	16.6	445	9.0	1195
3	Xerosis cutis	40	7.3	161	25.3	304	10.9	225	4.5	730
4	Viral warts	10	1.8	88	13.8	620	22.3	513	10.4	1,231
5	Urticaria	21	3.9	95	14.9	196	7.1	248	5.0	560
6	Seborrhoeic dermatitis	16	2.9	38	6.0	195	7.0	330	6.7	579
7	Bacterial infections	19	3.5	62	9.8	113	4.1	192	3.9	386
8	Dermato phytosis	9	1.7	52	8.2	92	3.3	181	3.7	334
9	Nevus	10	1.8	17	2.7	85	3.1	117	2.4	229
10	Atopic dermatitis	76	13. 9	64	10.1	63	2.3	23	0.5	226

Table 2: The distribution of the most common 10 diseases according to gender.

No	Disease	Gender: Male		Female		Total	
		n	%	n	%	n	%
1	Acne	1,067	11.8	1,439	15.9	2,506	27.7
2	Viral warts	637	7.0	594	6.6	1,231	13.6
3	Contact dermatitis	555	6.1	640	7.1	1,195	13.2
4	Xerosis cutis	360	4.0	370	4.1	730	8.1
5	Urticaria	241	2.7	319	3.5	560	6.2
6	Seborrhoeic dermatitis	193	2.1	386	4.3	579	6.4
7	Bacterial infections	217	2.4	169	1.9	386	4.7
8	Dermatophytosis	172	1.9	162	1.8	334	3.7
9	Nevus	97	1.1	132	1.5	229	2.5
10	Atopic dermatitis	127	1.4	99	1.1	226	2.5

Table 3: The rate and frequencies of the disease groups.

Diseases	n	%	Diseases	n	%
Infectious and parasitic diseases	**2,267**	**25.0**	**Bullous disorders**	**3**	**0.03**
Bacterial infections	386	4.7	Epidermolysis bullosa	3	0.03
Fungal infections	334	3.7	Pemphigus	0	0
Viral infections	1,526	16.8	Dermatitis herpetiformis	0	0
Parasitic infections	21	0.2	Bullous pemphigoid	0	0
Dermatitis and eczema	**2,237**	**24.7**	**Pigmentary disorders**	**191**	**2.1**
Contact dermatitis	1,195	13.2	Vitiligo	57	0.6
Atopic dermatitis	226	2.5	Pityriasis alba	76	0.8
Seborrhoeic Dermatitis	579	6.4	Postinflamatory hyperpigmentation	32	0.4
Nummular eczema	53	0.5	Ephelides	3	0.03
Neurodermatitis	21	0.2	Others	23	0.3
Nodular prurigo	2	0.02			
Pruritus	161	1.8			
Papulosquamous disorders	**234**	**2.6**	**Environmental disorders**	**123**	**1.4**
Psoriasis	112	1.2	Burn	11	0.1
Pityriasis rosea	103	1.1	Corns (Clavus)	88	1.0
Lichenoid dermatoses	14	0.2	Polymorphous light eruption	21	0.2
Pityriasis rubra pilaris	5	0.06	Ecchymose	3	0.03
Urticaria and erythema	**567**	**6.7**	**Vascular**	**24**	**0.3**
Urticaria	560	6.2	Pyogenic granuloma	20	0.2
Erythema nodosum	2	0.02	Hemangioma	4	0.04
Erythema multiforme	4	0.04			
Other erythematous conditions	1	0.01			
Genodermatoses	**6**	**0.07**	**Diseases of the oral cavity, salivary glands and jaws**	**39**	**0.4**
Neurofibromatosis	2	0.02			
Ichthyosis vulgaris	4	0.04			
			Recurrent oral aphthae	39	0.4
			Cheilitis	0	0
Skin disorders of the appendages	**544**	**6.0**	**Neoplasms**	**274**	**3.0**
Alopecia areata	116	1.3	Malign neoplasm	0	0
Rosacea	7	0.08	Benign neoplasm	274	3.0
Androgenic alopecia	33	0.4	Melanocytic naevi	229	2.5
Telogen effluvium	196	2.2	Other benign neoplasm of the skin	45	0.5
Hirsutism	9	0.1			
Hypertrichosis	9	0.1			
Hyperhidrosis	132	1.5			
Miliaria	42	0.5			
Acne	**2506**	**27.7**	**Xerosis cutis**	**730**	**8.1**
Nail disorders	**133**	**1.5**	**Not elsewhere classified**	**391**	**4.3**

Discussion

Among pediatric patients, the top three disease groups were, in descending order of incidence, acne (27.7%), infectious and parasitic diseases (n=2,267; 25.0%), dermatitis and eczema (n=2,237; 24.7%).

In a study by Colgecen et al. [3], acne (37.9%) was the most prevalent disease in the adolescence. In another prevalence study in which 10,115 subjects were included, acne vulgaris was reported with a rate of 17.82% (22). [4] In our study, the most frequent disease was acne (n=2506; 27.7%) which was also the

most frequent disease of the adolescence group (n=2297, 46.4%). The prevalence of acne was 11.8% - 25.2% (between the ages of 13 – 16 years) in previous studies conducted in Turkey (Table 4) [2]. Larsson and Liden [5] found the prevalence of acne 36.5% in the same age group. As a result of differences in patient selection and disease/patient classification, acne incidence shows variations among the studies. While most of the studies excluded 16-17 years old patients, in our study we included the patients who were younger than 18 years old.

Table 4: The rate of the disease in some previous studies among children in Turkey.

Disease	Present study, n=9,057[¥]	Ozcelic [4], n=10,115[¥]	Sacar [7], n=1,756[¥]	Can [6], n=850[¥]	Seckin [9], n=5,043[¥]	Colgecen [3], n=2,307[¥]	Akbas [2], n=4,025[¥]
Acne	2,506 (27.7)	1,803 (17.8)	66 (3.8)	30 (3.5)	796 (15.8)	472 (17.3)	279 (6.9)
Viral warts	1,231 (13.6)	1,015 (10)	148 (8.4)	37 (6.4)	-	- (11.2)	349 (7.8)
Contact dermatitis	1,195 (13.2)	1,071 (10.6)	78 (4.4)	102 (12)	366 (7.3)	194 (7.1)	626 (15.6)
Xerosis cutis	730 (8.1)	373 (3.7)	128 (7.3)	-	-	-	95 (2.4)
Urticaria	560 (6.2)	308 (3.0)	57 (3.2)	27 (3.2)	154 (3.1)	144 (5.3)	90 (2.2)
Seborrhoeic dermatitis	579 (6.4)	435 (4.3)	125 (7.1)	55 (6.5)	206 (4.1)	106 (3.9)	175 (4.3)
Bacterial infections	386 (4.7)	441 (4.4)	103 (5.9)	12 (1.4)	128 (2.5)	81 (3)	-
Dermatophytosis	334 (3.7)	436 (4.3)	46 (2.6)	31 (3.6)	195 (17.5)	88 (3.2)	-
Nevus	229 (2.5)	433 (4.3)	- (3.2)	25 (2.9)	122 (3.8)	46 (1.7)	-
Atopic dermatitis	226 (2.5)	408 (4.0)	140 (8)	110 (39.5)	665 (13.2)	172 (6.3)	318 (7.3)

[¥]:(n (%))

Akbas et al. [2] assessed that the prevalence of acne in the female (61%) was more frequent than male (39%). They found that there was a statistically significant difference between female and male pediatric patients [2]. Regarding the prevalence of acne, a female dominance (57.4%) was found in our study and it was also statistically significant.

Not surprisingly, the prevalence of acne was 0.2% (n = 1) in newborn and infant group, which increased with age and reached 46.4% in the adolescence group.

The prevalence of infectious and parasitic diseases was 25.0% in our study. It was similar to other studies from Turkey. In the previous studies from Turkey, the prevalence of infectious and parasitic diseases were found with a rate of 25.8% by Colgecen et al. [3], 13.4% in the study by Can et al. in Istanbul [6], 20.6% by Sacar et al. [7], 27.9% by Kacar et al. [8] , and also Seckin et al. 22% [9] in Tokat. Our result was not different from these results. Infectious diseases were reported to be the most commonly observed disease group with a rate of 11.4% in Northern India. [10] Again, Sardana et al. [11] was reported that the most commonly observed disease group (47.15%) among all subjects in the India. Additionally, infectious diseases were reported to be the most commonly observed disease group in the Ethiopia and Brazil [12-13]. Previous studies conducted in Ethiopian and Brazilian, parasitic infestations were found most commonly in children. [12-13] Crowded environments, inadequate hygiene, and low socioeconomic level may be the reason for the high frequency of infectious diseases in underdeveloped or developing countries.

Viral infections constituted the most commonly observed infectious skin diseases, similar to our study. This finding may be explained by reason of the fact that infectious skin diseases can easily be transmitted in crowded settings. [14]

Warts (13.7%) was the most common viral infection. The peak age group for warts was in the school age group (22.3%). There was statistically no significant difference between male (48.3%) and female (51.7%). Warts have a high prevalence in most studies, ranging from 4.6% to 17.5% [2,3,6-9]. Viral warts were found with a rate of 8.7% in the study of Akbas et al. [2] , with a rate of 11.2% in the study of Colgecen et al. [3] , with rate of 6.4%, in the study of Can [6] , with rate of 8.4% in the study of Sacar et al. [7] , with rate of 17.8% in the study of Kacar et al. [8] , and with rate of 12.1% in the stud of Seckin et al. [9] .

The most frequent diagnoses in dermatitis and eczema group were contact dermatitis. Contact dermatitis was more common in preschoolers and school groups than newborn, infant and adolescence groups. Contact dermatitis was 53.6% (n=640) in female and it was 46.4% in male. This result corresponds with findings of some similar studies [2, 9, 15-18] in Turkey. Heine et al. [19] assessed that the rate of sensitization in children and adolescents was similar to adults, increased contact with allergic or irritants substances with increasing age may be reason for the increased frequency found in preschoolers and school groups age groups.

Colgecen et al. [3] reported that atopic dermatitis occurred more frequently in infant and newborn and the prevalence was decreasing with the increasing age. Similarly, atopic dermatitis (13.9%) was the most common skin disease among the infant and newborn group in our study. It was less frequent in school (2.3%) and adolescence groups (0.5%). A male-to-female sex ratio of 1.28:1 was found in atopic dermatitis. The prevalence of this disease in the Turkish pediatric patients was found between 6.3% and 13% [3, 6, 9, 15].

There are some limitations in our study. The study was conducted in only one hospital. This is limiting the generalisability of the results. Another limitation of our study is the reliance on clinical records and not direct diagnosis.

In conclusion, acne, viral warts, and contact dermatitis were the three most common skin diseases that we detected in Eskisehir in pediatric population. In order to reveal the prevalence of pediatric skin diseases, further epidemiological studies are needed.

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Effects of resveratrol, catechin and epicatechin on rat phrenic nerve hemi-diaphragm

Sıçan frenik sinir hemi-diyaframına resveratrol, kateşin ve epikateşinin etkileri

Aslı Zengin Türkmen [1], Asiye Nurten [1]

Abstract

Aim: Resveratrol, catechin and epicatechin are known for their preventive effects of skeletal muscle atrophy, anti-fatique effects and enhancement of exercise capacity. In this study, we investigate the effects these antioxidants on muscle contraction by using rat phrenic nerve hemi-diaphragm preparations.
Methods: Twitch responses are investigated by giving direct and indirect and tetanic stimulation to rat phrenic nerve hemi-diaphragm preparations.
Results: Resveratrol and catechin facilitated twitch responses to the indirect stimulus in short duration and suppressed in long duration. The decrease in the responses to the direct stimulation by applying epicatechin (p=0.010) was significantly higher than the responses by giving indirect stimulation. Epicatechin significantly suppressed tetanic responses (p=0.005), but not resveratrol and catechin.
Conclusion: The results of the study show that these antioxidants have presynaptic and postsynaptic effects on neuromuscular junction. Resveratrol effective on muscle contraction via direct stimulus, catechin and epicatechin effects muscle contraction by direct and indirect stimulus.

Key words: Resveratrol, catechin, epicatechin, rat, antioxidant

Öz

Amaç: Resveratrol, kateşin ve epikateşin iskelet kas atrofisini engelleyen, yorgunluk karşıtı etkileri olan ve egzersiz kapasitesini artıran etkileriyle bilinmektedirler. Çalışmamızda, sıçan frenik sinir hemi-diyafram preparatı kullanılarak bu antioksidanların kas kasılmasına etkisi incelenmiştir.
Yöntemler: Kasılma yanıtları sıçan frenik sinir hemi-diyafram preparatına, doğrudan, dolaylı ve tetanik uyarılar verilerek araştırıldı.
Bulgular: Resveratrol ve kateşin uygulanması kısa sürede, dolaylı uyarılarla oluşan kasılma yanıtlarını kolaylaştırdı ancak uzun sürede baskıladı. Epikateşin uygulanması sonucunda doğrudan uyarılarla oluşan kasılma yanıtlarındaki azalma (p=0,010), dolaylı uyarılarla oluşan azalmadan anlamlı olarak daha fazlaydı. Resveratrol ve kateşin tetanik yanıtları etkilemezken, epikateşin anlamlı derecede baskıladı (p=0,005).
Sonuç: Çalışmanın sonuçları, bu antioksidanların nöromusküler kavşakta presinaptik ve postsinaptik etkileri olduğunu göstermektedir. Resveratrol kas kasılmasını doğrudan etkilemekte, kateşin ve epikateşin ise hem doğrudan hem de dolaylı olarak etkilemektedir.

Anahtar kelimeler: Resveratrol, kateşin, epikateşin, sıçan, antioksidan

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Introduction

Resveratrol is a well-known grape and wine polyphenolic component, synthesized in grape skin in response to infection. Numerous studies have reported resveratrol as a preventive agent against vascular disease, cancer, viral infections and neurodegenerative processes [1, 2].

Catechin and epicatechin are tea polyphenols and used in vascular, viral, gastrointestinal diseases [3]. Catechin has protective effects in skeletal muscle atrophy and down-regulate the expression of atrogenes, which are muscle atrophy related enzymes [4]. Another study showed that epicatechin enhances exercise capacity in mice [5].

Effect of different tea extracts were studied on neuromuscular junction [6, 7, 8, 9]. In a study with rat phrenic nerve hemi-diaphragm preparation, teaflavin fraction of black tea has facilitatory effect on indirect twitch responses [6]. Black tea extract also inhibits effect of boutulinum neurotoxin in mice [9]. Green tea extract (GTE) has facilitatory effect on indirect twitch responses at the lower concentrations, but its effect was suppressed at the higher concentrations [7].

With contraction of the hemi-diaphragm, free radicals may accumulate in the environment as time elapses. Superoxide and hydrogen peroxide (H_2O_2) are produced and released in skeletal muscle fibers [10]. Reactive oxygen species reduced muscle twitches by indirect stimulation; but, they have no effect by direct stimulation on rat phrenic nerve hemi-diaphragm preparation [11]. Also, recent studies have shown that free radicals cause muscle weakness in frogs [10]. Resveratrol suppress oxidative stress markers in aged mice with dietary administration [12]. Dietary resveratrol also slows age related structural changes in neuromuscular junction (NMJ) [13].

As well as the abundant effects of these polyphenols, there is not much data about the direct effects of catechin and epicatechin on the peripheral nervous system. Therefore, we aimed to investigate the effects of resveratrol and catechins on NMJ using rat phrenic nerve hemi-diaphragm preparation for this purpose. Then, the effects of these antioxidant molecules on muscle twitches were investigated by stimulating the phrenic nerve and hemi-diaphragm electrically.

Material and methods

This study was approved by the Animal Ethics Committees of Institute for Experimental Medicine, Istanbul University (Date: 01.03.2006, No: 10). All animal studies carefully conformed to the guidelines outlined in Interdisciplinary Principles and Guidelines for the Use of Animals in Research and Education from the New York Academy of Sciences.

Experiments were performed on adult male Wistar albino rats weighing 250-300 g provided by the Istanbul University, Aziz Sancar Institute for Experimental Medicine. Antioxidant molecules; resveratrol, (+) catechin and (-) epicatechin were purchased from Sigma and Aldrich Chem. Co., St. Louis, MO.

The phrenic nerve hemi-diaphragm preparations were isolated from decapitated rats, according to the method of Bulbring [14] (Figure 1). The preparation was suspended in a 20 ml organ bath containing Kreb's solution (133 mM NaCl, 4,9 mM KCl, 1,8 mM $CaCl_2$; 11,9 mM $NaHCO_3$; 0,7 mM NaH_2PO_4; 11mM Glucose) aerated with a mixture of 95% O_2 and 5% CO_2 at 37ºC. After resting for 15 minutes, preparation was fixed at the rib, while the hemi-diaphragm was fixed to the arm of isometric force transducer (MAY FDT 10-A, Grass Technologies; West Warwick, RI) with a tension of 2 g. The muscle was placed on platinum electrodes for direct stimulation and the phrenic nerve pulled into another electrode for stimulating the diaphragm indirectly.

Direct responses were studied on 13 preparations for resveratrol, 10 for catechin and 9 for epicatechin. For investigating indirect responses we used 11 preparations for resveratrol, 12 for catechin and 8 for epicatechin.

Supramaximal square wave pulses with 0.1 Hz frequencies and 0.3 ms duration were used for indirect stimulation, and 0.1 Hz 3 ms pulses were used for direct stimulation. For tetanic responses, the preparation was stimulated at 50 Hz and 3 ms indirectly. The responses were recorded by a polygraph (Grass Mode 7400 Physiological Recorder, Grass Technologies; West Warwick, RI) linked to the computer, and data were observed with "PolyVIEW v2.5 data analyze and acquisition system" (Astro-Med, West Warwick, RI).

All antioxidant molecules were dissolved in Kreb's solution containing 15% ethanol. We applied different doses of antioxidant molecules in a range of 25, 50, 62.5, 75, 100, 150 and 200 μM and found the optimal dose as 62.5 μM for resveratrol, 150 μM for catechin and 50 μM for epicatechin (Table 1). The effects of the antioxidant molecules were denoted as the changing percent of the responses, before and after the treatment. Contractions were recorded as baseline and 2.5th, 5th, 10th, 15th and 20th minutes.

Statistical analysis

All values were expressed as mean±standard error of mean (SEM). We first analyzed the responses by one-way ANOVA, and then, statistical significance of the direct and indirect responses were calculated by post-hoc analyses (Tukey's test). Significance of the tetanic responses was calculated by the Student's t-test. $p<0.050$ was considered statistically significant.

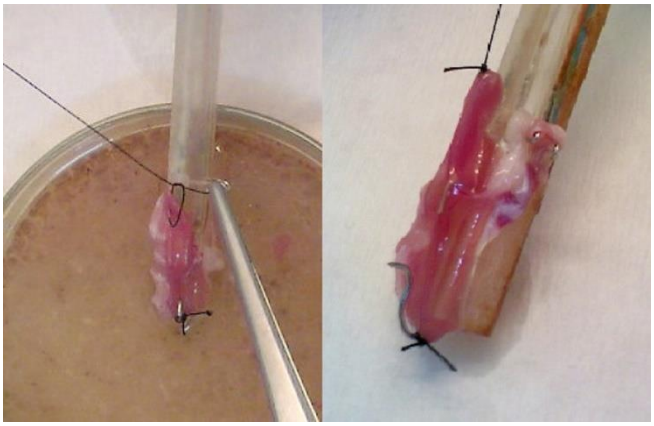

Figure 1. Isolated phrenic nerve hemi-diaphragm preparation placed at platinum electrodes.

Results

When we applied resveratrol, twitch responses gradually decreased in time by direct stimulation. Muscle contraction was reduced to 80.4±6.8% at 20th minutes, and this decrement was statistically significant from the basal value ($p=0.050$) (Figure 2).

Twitch responses to indirect stimulus increased to 114.2±7.7% at 2.5th minutes, then, non-significantly decreased to 92.1±5.3% at 20th minutes by applying resveratrol ($p=0.096$).

As shown in Figure 3, catechin administration significantly reduced muscle contraction by direct stimulation. Twitch responses decreased to 80.6±18.1% at 10th minutes ($p=0.050$), 80.5±6.1% at 15th minutes ($p=0.010$) and 76.9±15.8% at 20th minutes ($p=0.010$). These decrements were significantly different from the basal value.

When we applied catechin, twitch responses to indirect stimulus increased to 106.9±5.1% at 2.5th minutes, but decreased

to the basal values at 5th minutes. Catechin administration reduced twitch responses to 86.2±3.8% at 20th minutes. This decrement found statistically significant from the value at 2.5th minutes (p=0.010) and 5th minutes (p=0.050).

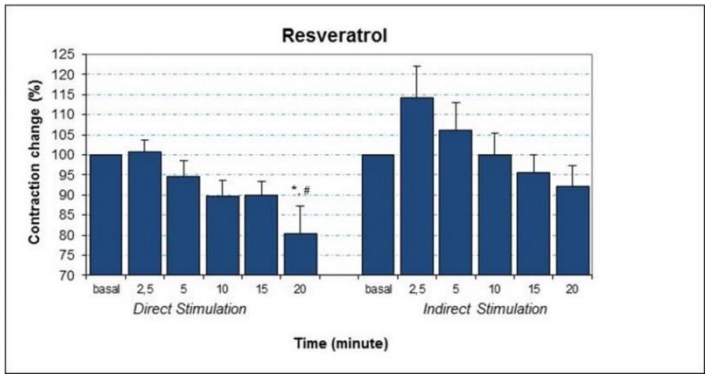

Figure 2. Timecourse of muscle contraction change after administration of resveratrol. Values represented as mean ± SEM. * compared with basal value, p=0.050, # compared with value at 2.5th minutes, p=0.050.

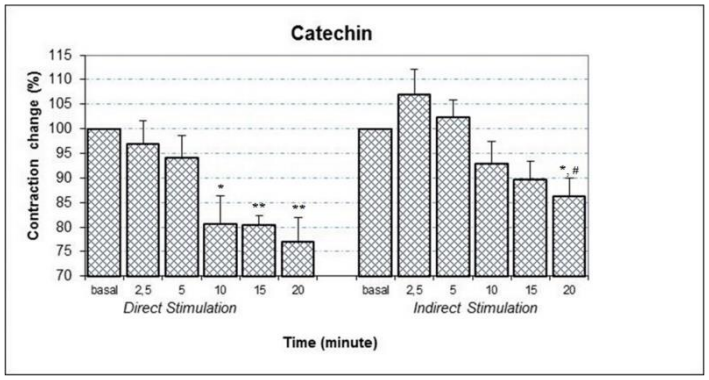

Figure 3. Effects of catechin on muscle contraction.
Values represented as mean ± SEM. * compared with basal value, p=0.050, ** compared with basal value, p=0.010,
compared with value at 2.5th minutes, p=0.010.

Epicatechin was significantly decreased the direct responses to 80.6±4.3% at 10th minutes (p=0.050), 76.6±4.3% at 15th minutes (p=0.010) and 70.9±6.7% at 20th minutes (p=0.010) compared to the basal values. Twitch responses to indirect stimulus decreased 88.4± 11.6% by applying epicatechin at 20th minutes. But, this is not statistically significant from the basal value (Figure 4).

When we stimulate the preparation tetanically, epicatechin significantly reduced muscle contraction at 20th minutes (p=0.005), there were no changes after resveratrol and catechin administration (Figure 5).

We used low concentrated ethanol as solvent of resveratrol and catechins. It was ineffective on direct, indirect and tetanic responses at the dose we used.

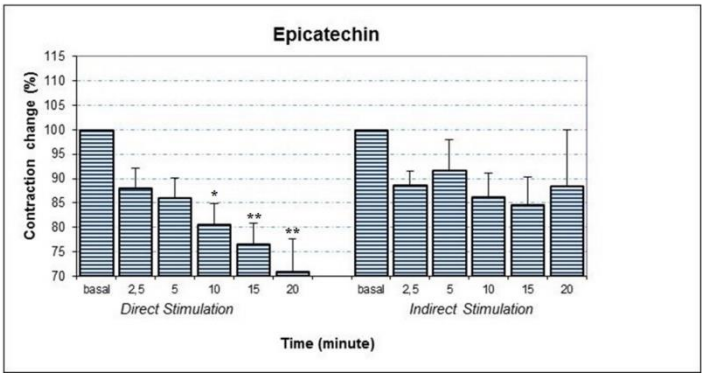

Figure 4. Effects of epicatechin on muscle contraction.
Values represented as mean ± SEM. * compared with basal value, p=0.050, ** compared with basal value, p=0.010.

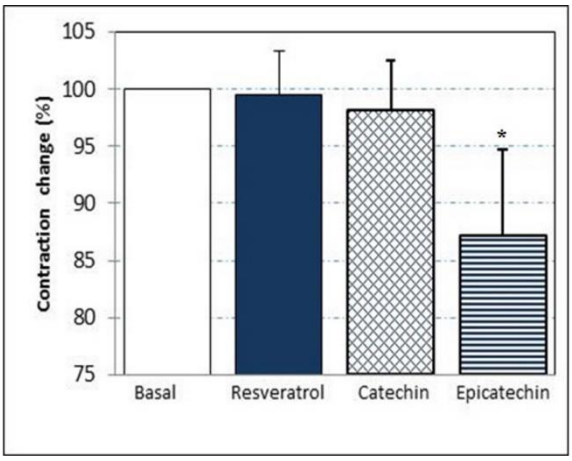

Figure 5. Effects of resveratrol, catechin and epicatechin administration on tetanic muscle contraction at 20th minutes. * compared with basal value, p=0.005.

Discussion

This study is designed to find out the effects of different antioxidants on the phrenic nerve hemi-diaphragm preparation. We showed the effects of resveratrol, catechin and epicatechin on muscle twitches to direct, indirect and tetanic stimulation. With direct stimulus, resveratrol, catechin and epicatechin reduced twitch responses gradually in time. We found no difference on tetanic responses in resveratrol and catechin treated NMJ preparations.

To our knowledge this is the first study that applies resveratrol, catechin and epicatechin on the phrenic nerve hemi-diaphragm preparation.

Resveratrol improved motor performance impairments by long-term application in stroke model in rats [15]. In our study, resveratrol enhanced muscle contraction in short duration, but suppresses in long term application. It can be argued that different results could be obtained in range of resveratrol concentrations.

Table 1. Dose response values of phrenic nerve hemi-diaphragm preparations to resveratrol, catechin and epicatechin.

Time (min) vs dose (µl)	Resveratrol								Catechin								Epicatechin							
	Direct Stimulation				Indirect Stimulation				Direct Stimulation				Indirect Stimulation				Direct Stimulation				Indirect Stimulation			
	5th	10th	15th	20th	5th	10th	15th	20th	5th	10th	15th	20th	5th	10th	15th	20th	5th	10th	15th	20th	5th	10th	15th	20th
25	99	115	98	40	62	78	81	-	66	80	23	20	76	100	44	40	70	72	70	78	85	92	92	103
50	101	100	97	95	92	90	93	106	58	68	50	44	36	49	49	64	***70***	***79***	***66***	***82***	***132***	***135***	***116***	***109***
62.5	***118***	***112***	***102***	***85***	***109***	***116***	***120***	***96***	68	80	98	98	103	98	98	94	82	89	78	82	105	97	86	112
75	97	105	102	107	97	95	96	91	106	113	106	107	98	-	92	105	69	79	65	85	127	121	112	115
100	100	102	51	110	68	85	87	78	105	107	109	116	105	92	89	90	70	75	70	76	120	113	115	110
150	105	90	96	100	75	84	89	85	***114***	***112***	***109***	***115***	***117***	***110***	***110***	***108***	90	95	98	90	100	95	95	-
200	65	65	-	50	70	90	90	95	33	26	32	34	117	107	107	113	88	102	-	-	65	80	50	62

The twitch responses were compared with basal values and denoted as changing percent in time. Selected dose for each antioxidant molecule indicated as bold and italic.

There are no studies showing the effects of catechin and epicatechin directly apply on neuromuscular junction. In a recent study, it has been shown that GTE, which have catechins abundantly, suppress muscle contraction in high doses [7]. GTE facilitates contraction by indirect stimulation in low concentrations, while suppressing the contraction response in high concentrations [7]. In another study, the exercise performance was better in mice, which fed with green tea rich diet [16].

According to results of our study, catechin, in the short term, increased the response to indirect stimuli similar to the effect of low concentrated GTE. However, catechin suppressed the twitch responses in extended time.

We found that catechin suppressed twitch responses by direct stimulation, but GTE has no effect. Different doses of GTE had no effects on contracted muscle, which directly stimulated by acetylcholine administration [7]. Green tea extract had no effect on the direct twitch responses, but the catechin exerted its effect alone. The presence of different compounds in the extracts besides of catechins, can also affect muscle contraction.

In summary, resveratrol suppressed muscle contraction by direct stimulus. Catechin and epicatechin minimize the muscle contraction with direct and indirect stimulus. Epicatechin reduced muscle contraction by tetanic stimulation.

In the light of these findings, we can say that the further studies on the effects of phenolic compounds on neuromuscular junction can give us more comprehensive information.

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Role of calcium–albumin ratio in severity of coronary artery disease assessed by angiographic SYNTAX score

Anjiyografik SYNTAX skoru ile değerlendirilen koroner arter hastalığı ciddiyetinde kalsiyum-albumin oranının rolü

Yakup Alsancak [1], Serkan Sivri [2], Serdal Baştuğ [3], Engin Bozkurt [3]

Abstract

Aim: Numerous studies have reported a relationship between serum calcium or albumin levels and acute coronary syndromes and coronary artery disease. The present study investigated the relation between serum albumin, calcium or albumin-corrected calcium levels or calcium/albumin ratio (CAR) and extensiveness and severity of atherosclerosis.

Methods: This prospective study included patients with non-ST elevation myocardial infarction (NSTEMI, n=120) and a control group (n=109). We used the SYNTAX score to evaluate the association between CAR and severity and extent of coronary artery disease.

Results: There were higher, but statistically nonsignificant, levels of calcium in patients with NSTEMI (p=0.058). However, serum albumin-corrected calcium levels were significantly higher in NSTEMI group (p=0.001). Yet, albumin levels did not differ between the groups (p=0.093). CAR and corrected calcium–albumin ratio (cCAR) were significantly higher in NSTEMI group (p=0.001). A positive correlation existed between CAR (r=0.235, p=0.010), cCAR (r=0.259, p=0.004), and SYNTAX score, whereas albumin and SYNTAX score (r=−0.259, p=0.004) showed a negative correlation.

Conclusion: Calcium/albumin ratio has been found to be associated with an increased coronary atherosclerotic burden as calculated by SYNTAX score. Further large-scale studies are warranted to confirm our findings.

Keywords: Non-ST elevation acute myocardial infarction, SYNTAX score, calcium-albumin ratio

Öz

Amaç: Serum kalsiyum ve albumin seviyeleri ile akut koroner sendromlar ve koroner arter hastalığı arasındaki ilişki birçok çalışmada gösterilmiştir. Sunulan çalışmada, serum albumin, kalsiyum, albumin-düzeltilmiş kalsiyum seviyeleri ve kalsiyum/albumin oranı (KAO) ile aterosklerozun yaygınlık ve ciddiyetinin ilişkisi araştırılmıştır.

Yöntemler: Bu prospektif çalışmaya non-ST elevasyonlu miyokard infarktüsü olan hastalar (NSTEMI, n=120) ve kontrol grubu (n=109) dahil edildi. KAO ile koroner arter hastalığı yaygınlık ve ciddiyetinin ilişkisini değerlendirmek için SYNTAX skoru kullanıldı.

Bulgular: NSTEMI hastalarında istatistiksel anlamlı olmadan daha yüksek kalsiyum seviyeleri vardı (p=0.058). Buna rağmen serum albumin-düzeltilmiş kalsiyum seviyeleri istatistiksel anlamlı olarak NSTEMI grubunda daha yüksekti (p=0.001). Albumin seviyeleri ise gruplar arasında farklılık göstermedi (p=0.093). KAO ve düzeltilmiş kalsiyum/albumin oranı (dKAO), NSTEMI grubunda anlamlı olarak daha yüksekti (p=0.001). KAO (r=0.235, p=0.010) ve dKAO (r=0.259, p=0.004) ile SYNTAX skoru arasında pozitif korelasyon mevcut iken, albumin ile SYNTAX skoru (r=−0.259, p=0.004) arasında negatif korelasyon görüldü.

Sonuç: Kalsiyum/albumin oranı, SYNTAX skoru ile hesaplanan artmış koroner aterosklerotik yük ile ilişkili bulunmuştur. Bulgularımızı doğrulamak için daha geniş çaplı çalışmalar gereklidir.

Anahtar kelimeler: Non-ST elevasyonlu akut miyokard infarktüsü, SYNTAX skoru, kalsiyum-albumin oranı

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Introduction

Increasing incidences of atherosclerotic cardiovascular diseases have become a growing source of global disease burden, thereby leading to a significant rise in global morbidity and mortality [1]. Medical professionals, with a keen interest in the subject, have been working on several different scoring systems and laboratory parameters to obtain an estimate of the prognosis and severity of the disease. The SYNTAX score is one of the most widely used angiographic grading tools for determining the complexity and severity of coronary artery disease (CAD). Many earlier studies demonstrate a higher SYNTAX score as an independent predictor of cardiovascular mortality and morbidity in patients with acute coronary syndromes (ACS) [2-4]. A recent, large-scale study estimated the levels of serum albumin in patients with ACS to be inversely proportional to the SYNTAX score and in-hospital mortality [5]. Moreover, various studies report hypoalbuminemia not only to be a predictive marker for an increased risk of developing CAD, but also a prognostic factor for heart failure, stroke, and myocardial infarction [6-8]. Several studies have hypothesized different mechanisms for the effect of albumin on the atherosclerotic cardiovascular disease. At the first place, ACS that represent an inflammatory process are accompanied by changes in albumin, a negative acute-phase reactant [9, 10]. Secondly, albumin has been found to inhibit the activation and aggregation of platelets [11]. It acts as a specific inhibitor of human endothelial apoptosis and serves as a major antioxidant in serum, where it plays a crucial role in ligand binding and free-radical trapping [12, 13]. In addition, albumin is the major binding protein for calcium, which has a significant role in cardiovascular diseases, including platelet activation, coagulation, and cardiac contraction. In short, calcium is found in three different forms in the plasma: (1) as free or ionized form, which is the physiologically important form (50%), (2) linked to albumin (40%), and (3) as a soluble complex (10%) [14]. Thus, it can be speculated that the levels of serum calcium and albumin are interrelated. Therefore, the level of calcium can not be estimated without evaluating the level of serum albumin during a laboratory test. The lower levels of calcium reported in patients with ST-elevation myocardial infarction (STEMI), like serum albumin levels, are considered to be a predictive factor for in-hospital mortality [15]. In another study, authors reported similar findings and suggested that inclusion of serum calcium in the GRACE (Global Registry of Acute Coronary Events) scoring system may further help in the more precise prediction of the risk [16]. Though many studies have demonstrated an inverse relationship between serum calcium or albumin levels and ACS and CAD, a few have also shown a positive relationship between these factors. Contrary to above, some studies report no relationship between these players [17-21]. Therefore, the dilemma whether serum calcium and albumin levels are risk factors for CAD persists.

The current study investigated the relationship between serum albumin, calcium or albumin-corrected calcium levels and especially, two interdependent variables, calcium to albumin ratio (CAR), and the extensiveness and severity of atherosclerosis in CAD.

Material and methods

Study Population

This study was designed in a prospective case-control manner. The local ethical committee approved the experimental protocol Yıldırım Beyazıt University Ethical Committee with a number of 82/2015 with confirmation of Helsinki declaration. All patients were informed about the aims and protocol of the study and written informed consent was obtained.

The study population consisted of 120 patients, with non-ST elevation myocardial infarction (NSTEMI), consecutively admitted to Atatürk Research and Training Hospital from January till December 2016 and 109 healthy subjects with a normal coronary angiography, normal treadmill stress test, myocardial perfusion scintigraphy or dobutamine stress echocardiography. The latter served as the control group. Patients with chronic severe renal or liver diseases, any disease of parathyroid glands, known calcium homeostasis disorders, hematologic disorders, an active malignancy, acute or chronic inflammatory disease and patients on thyroid replacement or immunosuppressive or hormone replacement therapy and who were administered drugs that affect bone or calcium metabolism, such as diuretics, corticosteroids, calcium or vitamin D supplements were excluded from the study. Hypertension was defined as repeated blood pressure measurements >140/90 mm Hg or history of a treatment with antihypertensive drugs. Diabetes mellitus was defined as fasting blood glucose >126 mg/dL, blood glucose >200 mg/dL at any time, or any patient treated with diet, oral medications, or insulin. Patients were designated as ‘‘current smokers’’ if they reported a history of active smoking in the last 6 months. Coronary angiography was performed for all patients who presented with NSTEMI. For the calculation of SYNTAX score, all coronary lesions causing ≥ 50% stenosis in vessels with a diameter of more than 1.5 mm were included. The SYNTAX score was calculated as per the latest online version of the software available on the website (http://www.SYNTAXscore.com). Different parameters were used for the calculation of the score including coronary dominance, number of lesions, segments included per lesion, the presence of total occlusion, bifurcation, trifurcation, aorto-ostial lesion, severe tortuosity, calcification, thrombus, diffuse/small vessel disease, and lesions measuring >20 mm. Individual SYNTAX scores using patient characteristics were analyzed by two to three experienced interventional cardiologists who were blinded for the study protocol. Patients were divided into 2 groups based on the SYNTAX score as high (≥ 23) or low score (<23) [2]. All patients underwent echocardiographical examination, and the left ventricular ejection fraction was calculated using the modified Simpson method [22].

Laboratory examinations

After 12 hour of fasting period, blood samples for hematologic and biochemical tests were collected. Serum albumin and calcium levels were analyzed at the first contact in the emergency room. Serum levels of fasting plasma glucose, lipid parameters, creatinine, and hematological values were determined using the standard methods. The serum high sensitive troponin T (hs-TnT) levels were measured using the Elecsys® Troponin T-high sensitive systems with a reference range of 3 to 14 pg/mL (Roche Diagnostics Corporation; Manheim, Germany). Serum albumin and calcium levels were calculated using the COBAS INTEGRA Albumin Gen. 2/cobas c systems (Roche Diagnostics Corporation; Manheim, Germany). We used a reference range of 3.5 to 5.2 g/dL and 8.8 to 10.2 mg/dL for albumin and calcium tests, respectively. The corrected serum calcium levels (cCalcium) were calculated using the most commonly used formula in clinical practice: Corrected calcium= measured total calcium (mg/dL) + 0.8 (4.0 − serum albumin [g/dL]) [23]. By using cCalcium levels, corrected calcium to albumin ratio (cCAR) was also calculated.

Statistical Analysis

The data collected during the research were analyzed using the SPSS 15.0 statistical package program (SPSS Inc., Chicago, IL, United States). Descriptive statistics were depicted

as mean ±standard deviation or median (inter-quartile range [IQR]) for continuous variables, and as the number of cases (n) and percentages (%) for categorical variables. Normality distribution was evaluated using the Kolmogorov–Smirnov test. Baseline characteristics were compared with the independent sample t-test, Mann–Whitney U test, chi-square test, or Fisher's exact test (wherever applicable). Spearman's correlation test was used to assess the correlation between calcium, albumin, CAR, and the SYNTAX score. The independent predictors of a high SYNTAX score were determined using the logistic regression analysis, which was performed as a multivariate analysis on parameters with statistically significant ($p<0.05$) differences as observed in univariate analysis. A p value less than 0.05 was considered to be statistically significant.

Results

The demographic features and laboratory parameters of the study population are shown in Table 1.

Table 1: Baseline clinical characteristics and laboratory parameters of the study population.

Variables	NSTEMI group (n=120)	Control group (n=109)	p
Age (years)¥	59.96 ±12.07	57.73 ±10.5	0.139
Hypertensionβ	67 (55.80)	57 (52.29)	0.343
Diabetesβ	35 (29.20)	30 (27.52)	0.449
Smokingβ	62 (51.66)	47 (43.11)	0.069
Fasting blood glucose (mg/dL)µ	116.50 (99–166)	93 (87–107)	0.001
eGFR (mL/min)µ	78 (48–110)	81(48–114)	0.543
Creatinin (mg/dl) ¥	1.1±0.31	0.9±0.22	0.041
Total cholesterol (mg/dL)¥	188.58 ±40.70	199.94 ±41.18	0.037
HDL (mg/dL)µ	40 (32.50–49)	45 (36–55)	0.002
LDL (mg/dL)µ	110.92 ±33.84	121.91 ±34.97	0.016
TG (mg/dL)µ	133 (82–196.50)	134 (101–187)	0.518
WBC (/mL)µ	8.39 (6.80–10.10)	7.60 (6.40–9.28)	0.006
Platelet × 1000 K/uLµ	217.50 (176.50–261)	257 (220–312)	0.001
MPV (fL)µ	9.30 (8.50–10.30)	10.60 (10.10–11.50)	0.001
hsTroponin T (pg/mL)µ	29 (8–162.5)	0.07 (0.01–0.15)	0.001
CK-MB (ng/mL)µ	2.90 (1.80–8)	-	-
LVEF (%)µ	60 (45–65)	65 (55–65)	0.001
Calcium (mg/dL)µ	9.60 (9.30–9.80)	9.51 (9.15–9.73)	0.058
cCalcium (mg/dL)µ	9.25 (9.06–9.52)	9.10 (8.82–9.28)	0.001
Albumin (gr/dL)µ	4.40 (4.17–4.60)	4.49 (4.20–4.67)	0.093
Calcium Albumin Ratioµ	2.19 (2.10–2.30)	2.11 (2.01–2.24)	0.001
cCalcium Albumin Ratioµ	2.10 (2–2.26)	2.01 (1.91–2.19)	0.001
SYNTAX score¥	9.19 ±9.72	-	-

¥: mean ± standard deviation, β: n (%), µ: median-IQR
NSTEMI: non-ST elevation myocardial infarction, GFR: Glomerular filtration rate, HDL: high-density lipoprotein cholesterol, hs: high sensitive, LDL: low-density lipoprotein cholesterol, TG: triglyceride, WBC: white blood cell, MPV: mean platelet volume, LVEF: left ventricular ejection fraction, CK-MB: creatinine kinase-myocardial band, IQR: interquartile range.

The mean age of the study population (n=229) was 59.01 ±11.35 years. Diabetes mellitus, hypertension, and smoking were reported to be more common in NSTEMI group, but not statistically significant. On the contrary, the levels of fasting blood glucose (p=0.001), creatinine (p=0.041), white blood cell (p=0.006), and high-sensitive cardiac troponin (hsTnT) (p=0.001) were significantly higher in patients belonging to NSTEMI group, as compared to control group. Among calcium and albumin serum levels, those of calcium were higher, but not statistically significant, in NSTEMI group (p= 0.058), whereas serum albumin-corrected calcium levels were statistically significantly higher in NSTEMI group (p=0.001). However, albumin levels did not differ between the groups (p= 0.093). Though CAR and cCAR were significantly higher in NSTEMI group, cCAR was found to be statistically more significant than CAR (p=0.001 and p=0.001, respectively). Median SYNTAX score was found to be 7 (IQR: 0–14) in NSTEMI group. CAR and cCAR demonstrated statistical difference among the subgroups with patients having a SYNTAX score < 23 (n=104) and ≥ 23 (n= 16, p= 0.048). Furthermore, as per the correlation analysis, the relationship between calcium, corrected calcium, and SYNTAX score (p=0.083 and p=0.504, respectively) was found to be non-significant. CAR (r=0.235, p=0.010), cCAR (r=0.259, p=0.004), and SYNTAX score were found to be positively correlated, whereas albumin and SYNTAX score (r=−0.259, p=0.004) displayed a negative correlation (Figure 1, 2). Univariate logistic regression analysis depicted albumin, cCalcium, CAR, cCAR, age, and ejection fraction to be significantly associated with a higher SYNTAX score (Table 2).

However, the multivariate regression analysis predicted only ejection fraction to be an independent predictor for high SYNTAX scores (p=0.001).

Table 2: Logistic regression analysis to determine the highest SYNTAX score.

Variables	Univariate Model				Multivariate Model			
	OR	95% CI		p	OR	95% CI		p
		Lower	Upper			Lower	Upper	
Age	1.092	1.035	1.152	0.001				
LVEF	0.874	0.815	0.937	0.001	0.866	0.797	0.924	0.001
HDL	1.022	0.990	1.054	0.177				
Hypertension	1.153	0.685	1.941	0.591				
Diabetes mellitus	1.136	0.637	2.027	0.549				
Albumin	0.103	0.025	0.430	0.002				
cCalcium	1.340	1.020	1.760	0.035				
Calcium albumin ratio	1.210	1.080	1.360	0.001				
cCalcium albumin ratio	1.022	1.012	1.033	0.001				

CI: Confident interval, LVEF: left ventricular ejection fraction, HDL: high density lipoprotein cholesterol, cCalcium: corrected calcium

Discussion

The current study established the presence of higher levels of serum calcium, corrected calcium levels and CAR in patients with NSTEMI than the healthy subjects. Also, this ratio was found to be associated with an increased coronary atherosclerotic burden as calculated by SYNTAX score. A significant correlation was reported between erum calcium and cCalcium levels and albumin ratio and SYNTAX score; however, the multivariate logistic regression model revealed CAR and cCAR not to be independent predictor factors for a high SYNTAX score. This study failed to demonstrate a positive or negative association between calcium and albumin levels in the patients with NSTEMI and those in control group.

The development and progression of atherosclerosis occur by endothelial dysfunction, platelet activation, oxidative stress, and inflammation, which are well-known mechanisms for atherosclerosis [24, 25]. Several studies have been conducted to investigate the effect of novel inflammatory markers on the relationship between inflammation and atherosclerosis during the

past decade. These findings indicated inflammation to play a significant role throughout the atherosclerotic process right from

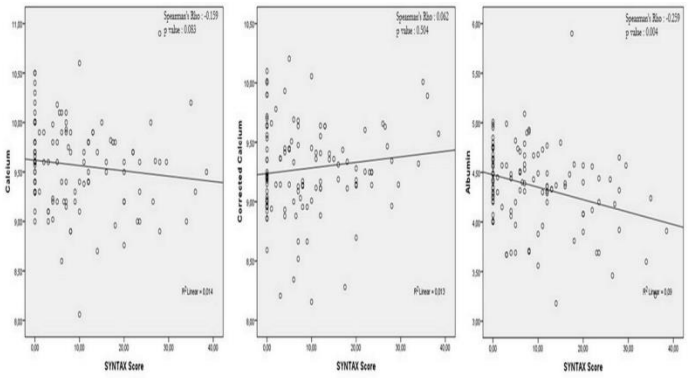

Figure 1. Correlation analysis with calcium, corrected calcium, albumin levels and SYNTAX score.

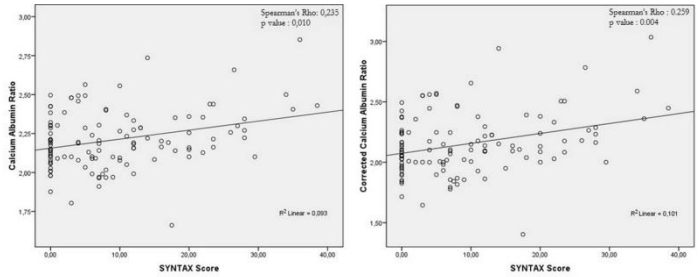

Figure 2. Correlation analysis with calcium albumin ratio, corrected calcium albumin ratio, and SYNTAX score.

the initiation to progression, activation of plaque, development of ACS as well as in the expansion of infarction [10, 26, 27]. Serum albumin, a negative acute-phase protein, is insufficiently produced by the liver during inflammation [28]. Similarly, several other studies have established low levels of albumin to be related to an increased risk of cardiovascular mortality and morbidity [29, 30]. Oduncu et al. [31] confirmed that low levels of serum albumin in patients admitted with NSTEMI were associated with a poor post-procedural myocardial reperfusion, a poor post-procedural left ventricular ejection fraction as well as complications related to infarct. They also found lower levels of serum albumin to be related to a higher cardiac and non-cardiac mortality, complex heart failure, stroke, and re-infarction during a long-term follow-up period (40 months). In another study, Celik et al. [32] demonstrated a lower serum albumin level, on admission, to be related to the development of restenosis in patients with bare-metal stents. On the other hand, studies exist that indicate lower serum albumin levels not to be associated with an increase in the probability of prevalent carotid atherosclerosis, both in males and females [19]. The present study reported a lower, although statistically nonsignificant, serum albumin level in the NSTEMI group. The finding could be attributed to following mechanisms: (1) Since the average age of the patients included in the study was low, a lower rate of chronic malnutrition or comorbid diseases could be anticipated. (2) Rigid inclusion criteria of the study might have influenced the results. (3) Inflammation is a well-known cause of atherosclerosis. As a high SYNTAX score is associated with the extent and severity of atherosclerosis, the lower intensity of inflammation in our study population could be explained by the lower average SYNTAX score (median=7) in NSTEMI group. These factors must have avoided the decrease of serum albumin concentration to statistically significant rates. As albumin is the major binding protein for calcium, several albumin-based standardization formulas have been recommended for the calculation of serum calcium levels. Recently published studies have demonstrated hypocalcemia to be a predictor of increased in-hospital mortality in patients with severe CAD [15, 16]. However, other studies have reported a high serum calcium concentration to be an independent predictor of incidence of CAD [33]. Also, a close association is known to exist between the serum calcium levels and traditional cardiovascular risk factors, such as hyperlipidemia, hypertension, and hyperglycemia [34, 35]. Another previously published study confirmed a significant linear relation between serum calcium level and blood pressure and the level of triglycerides. The same study also proved the level of cholesterol to increase with an elevation in the serum calcium levels [35]. Furthermore, serum calcium levels are higher in non–insulin dependent diabetes mellitus [36]. In the present study, though higher serum calcium levels were found in NSTEMI group than in control group, these were not statistically significant. In addition, serum albumin-corrected calcium level was significantly higher in NSTEMI group. It has been demonstrated that elevated calcium levels and the higher calcium/albumin ratio, emerged as a novel parameter, were strongly associated with all-cause mortality in patients with stable CAD [37]. It can be assumed that the results of this study support our findings. But, we need more data to confirm the association between all cause mortality and CAR in patients with NSTEMI.

Since the association between the levels of both serum calcium and albumin and atherosclerotic cardiovascular diseases is still in debate and has been shown to be positively correlated, we hypothesize that a higher calcium or cCAR may reflect their association with the presence and extensiveness of CAD more accurately than albumin and calcium alone. Given that the metabolism of albumin and calcium is closely related to each other, their ratio may predict the outcome of CAD more accurately. One of the mechanisms of action of albumin on the development of CAD could be its influence on calcium metabolism. To conclude, we believe that better and efficient results on the association between CAD and serum calcium could be obtained by the estimation of albumin-corrected calcium levels.

The most significant limitation of the present study was the insufficient sample size. Besides, data obtained did not allow us to evaluate the prognostic value of CAR or cCAR on adverse cardiovascular outcomes. Furthermore, the study lacked the comparison of CAR or cCAR with other inflammatory markers, such as CRP, hsCRP, fibrinogen, or IL 6, owing to the retrospective design of the study. Another important lack in the study was that all of the control group was not selected from patients with normal coronary arteries after coronary angiography. A low number of patients with a SYNTAX score of over 22 might have also reduced the strength of the study. And lastly, the analysis did not include concomitant medications of patients and serum levels of vitamin D or parathyroid hormone levels.

In conclusion, the present study is the first of its kind to demonstrate the relation between CAR or cCAR and atherosclerotic CAD. There is a need for an easily accessible and cost-effective biomarker to determine the disease activity in patients with atherosclerosis. In this context, the results of the study are clinically significant owing to the easily available estimation of CAR/cCAR, wider use, and being a relatively economical method for the determination of inflammatory status of body when compared to most other inflammatory markers. Further large-scale studies and investigations are warranted to authenticate the findings of this study on the atherogenic role of CAR/cCAR.

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Renoprotective potential of quercetin in experimental diabetic nephropathy: assesing antiapoptotic and antioxidant effects

Deneysel diyabetik nefropatide quercetin'in renoprotektif potansiyeli: antiapoptotik ve antioksidan etkilerin değerlendirilmesi

Matem Tunçdemir [1], Eda Büyükçolpan Mirzataş [1], Hafize Uzun [2]

Abstract

Aim: We investigated renoprotective and anti-apoptotic effects of quercetin, a potent bioflavonoid, by evaluating expression of apoptosis-regulatory genes that contribute to the kidney damage caused by diabetes in rats.

Methods: Rats were divided into 4 groups; Control, STZ-induced diabetic, STZ-induced diabetic+Quercetin and Quercetin control. Values of fasting blood glucose, body weight and urine microalbuminuria measured. Immunohistochemistry was performed using bax, bcl-2, caspase-3 antibodies. For apoptosis detection, TUNEL method was applied. Advanced oxidation protein products (AOPP), protein carbonyl oxidation (PCO), malondialdehyde (MDA) and superoxide dismutase (SOD) activity were measured in homogenized kidney tissues.

Results: Blood glucose and microalbuminuria levels were significantly decreased in quercetin-treated diabetic group compared to the untreated-diabetic group (p=0.020 and p=0.003; respectively). MDA, AOPP and PCO levels were significantly decreased (p=0.001, p=0.0001 and p=0.0005; respectively); however, SOD activity were found to increase in quercetin-treated diabetic group (p=0.005). Immunostaining of bcl-2, bax and caspase-3 was decreased compared to the untreated-diabetic group. Apoptotic cells especially increased in the kidney tubuli of untreated-diabetic group and on the contrary, a significant decrease was observed in the group that received a quercetin treatment (p=0.0001).

Conclusion: Our results revealed that antiapoptotic effects of quercetin, which has predominantly antioxidant effects, may be useful in reducing effects of diabetic complications and preventing new complications.

Key words: Experimental diabetic nephropathy, quercetin, kidney, apoptosis, oxidative stress

Öz

Amaç: Sıçanlarda diyabetin neden olduğu böbrek hasarına katkıda bulunan apoptoz düzenleyici genlerin ekspresyonunu değerlendirerek güçlü bir biyoflavonoid olan quercetin'in renoprotektif ve antiapoptotik etkilerini araştırmak.

Yöntemler: Sıçanlar 4 gruba ayrıldı; Kontrol, STZ-diyabetik, STZ-diyabetik + Quercetin ve Quercetin kontrol. Açlık kan şekeri, vücut ağırlığı ve idrar mikroalbüminüri değerleri ölçüldü. İmmünohistokimya bax, bcl-2, kaspaz-3 antikorları kullanılarak gerçekleştirildi. Apoptoz tespiti için TUNEL yöntemi uygulandı. Homojenize böbrek dokularında ileri oksidasyon protein ürünleri (AOPP), protein karbonil oksidasyon (PCO), malondialdehid (MDA) ve süperoksit dismutaz (SOD) aktivitesi ölçüldü.

Bulgular: Tedavi edilmeyen diyabetik gruba kıyasla, quercetin uygulanan diyabetik grupta kan şekeri ve mikroalbüminüri düzeyleri anlamlı olarak azalmıştı (sırasıyla; p=0,020, p=0,003). MDA, AOPP ve PCO seviyeleri anlamlı olarak azaldı (sırasıyla; p=0,001, p=0,0001, p=0,0005), ancak SOD aktivitesinin quercetin uygulanan diyabetik grupta arttığı tespit edildi (p=0,005). Quercetin uygulanan diyabetiklerde, tedavi edilmemiş diyabetik gruba kıyasla bcl-2, bax ve kaspaz-3'ün immün boyanması azaldı. Tedavi edilmeyen diyabetik grubun böbrek tübüllerinde apoptotik hücrelerde belirgin bir artış gözlenirken, quercetin uygulanan diyabetik grupta belirgin bir düşüş gözlendi (p=0,0001).

Sonuç: Antioksidan etkileri olan quercetin'in antiapoptotik etkilerinin, diyabetik komplikasyonların etkilerini azaltmada ve yeni komplikasyonları önlemede yararlı olabileceği sonucuna vardık.

Anahtar kelimeler: Deneysel diyabetik nefropati, quercetin, böbrek, apoptoz, oksidatif stres

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Introduction

Diabetic nephropathy (DN), which is also the chronic complication of Diabetes Mellitus (DM), is characterized by glomerular hypertrophy, proteinuria, decreased glomerular filtration and renal fibrosis that lead to renal dysfunction [1].

Streptozotocin (STZ) is frequently used in different doses to induce DM in experimental animals and the mechanisms of diabetes are attempted to elucidate [2]. STZ has a toxic effect on pancreatic β cells. It gives rise to renal tissue dysfunction and damage caused by insulin deficiency and hyperglycemia; and thereby, it generates the diabetic nephropathy table observed in clinics [2, 3]. STZ, as a free radical source, leads to DNA damage and afterwards, cell death [4]. It is known that free oxygen radicals take place in the pathogenesis of renal damage. It is reported that diabetes accelerates formation of reactive oxygen species (ROS) and increased oxidative stress is associated with irreversible renal damage and DN pathogenesis [5].

Apoptosis, which means programmed cell death, is a physiological process that plays important roles in both persistence of cellular homeostasis and both proliferation and differentiation of cells. The damaged cells or the ones with danger potential are destroyed with apoptosis. It has been reported in different types of cells that increased levels of ROS cause apoptosis [6]. Low levels of ROS function as "redox messengers" that is important for cellular signaling and homeostasis. It is revealed that increased ROS level in kidneys is associated with apoptosis in addition to progression and development of diabetic nephropathy [7]. It is considerably important to perceive changes in apoptosis related genes, oxidative stress biomarkers and antioxidant enzyme activity levels for better understanding of apoptotic mechanism [8].

It is suggested that antioxidants have protective effects against apoptosis [9] Flavonoids, which belong to polyphenol group, have strong antioxidant effect and they are also commonly used for treatment of several diseases. Quercetin (3,5,7,3',4'-pentahydroxyl flavon), which has many useful pharmacological effects like anti-hypertensive, anti-ischemic, anti-carcinogenic, anti-thrombotic, anti-inflammatory in addition to its anti-oxidant effect, is one of the important natural polyphenolic flavonoids [6, 10]. It is also indicated that quercetin has beneficial effects on prevention of ischemia reperfusion [9] and diabetes induced renal damage [11].

We aimed comparatively to investigate the renoprotective and antiapoptotic effects of quercetin by evaluating the expression of apoptosis-regulatory genes that contribute to the kidney damage caused by diabetes in rats.

Material and methods

Animals and protocols

All experiments were approved by the Istanbul University Cerrahpasa Faculty of Medicine Ethics Committee for Animal Experiments (project no:2015/36), and followed the NIH Guide for the Care and Use of Laboratory Animals. Wistar-type-albino male rats weighing approximately 310-410 g were purchased from Istanbul University, Aziz Sancar Institute of Experimental Medicine.

All groups, except the control groups, received a streptozotocin injection (STZ; Sigma, St.Louis, MO,USA,50 mg/kg, freshly dissolved in 0.9 % sodium chloride, i.p.). On the 3rd day after the STZ injection, the rats developing diabetes (above blood glucose levels of 350 mg/dL) [3] were divided into two groups. The first group consisted of untreated-diabetic rats (n=8). Second group of diabetic rats was treated with quercetin (20 mg/kg/day, diluted in 4% ethanol, i.p., 30 days, sc-206089B, Santa Cruz Biotechology (SCBT), n=8). Third control group was treated with quercetin (20 mg/kg/day, n=8). Fourth group was the control group consisted by the non-diabetic rats (for sham injection, 0.9 % sodium chloride, i.p., (n=8). The animals had free access to standard rat chow and drinking water. At the end of the experimental period (31st day), the animals were sacrificed under anesthesia (ketamine–xylazine), and kidney tissue samples removed for histological and biochemical examinations.

Blood glucose levels of all groups were measured using Blood Glucose test strips (Blood Glucose Test-Strip,Taiwan) with a glucometer (eBsensor Blood Glucose Monitoring System, Taiwan eB-G model) in samples obtained from the tail vein. At the 1st,15th, and 30th day of the experiment, all rats from each group were housed in metabolic cages (24-h) for urine obtain. Daily urine volume and microalbuminuria levels were measured from collected urine. Microalbuminuria was measured using Micral-test strips (DIRUI, Urine Analyzer, H11-MA urine strips). Body weights of all animals were measured in a weekly manner. Rats were dissected to obtain the kidneys. The right kidneys from each rat were weighed and recorded.

Biochemical measures

The kidney tissues were homogenized in a fourfold volume of phosphate buffer solution (PBS) using a homogenizer (Next Advance Bullet Blender Storm 24). The homogenate was centrifuged at 3,000×g for 10m to remove debris. Clear upper supernatant was taken, and tissue analyses [(malondialdehyde (MDA), superoxide dismutase (SOD), the advanced oxidation protein products (AOPP), protein carbonyl oxidation (PCO)] were carried out. All procedures were performed at +4°C throughout the experiments.

Measurement of tissue MDA levels: MDA levels were determined as previously described by Ohkawa et al. [12] with a minor modification. The reaction mixture was prepared by adding 0,25ml homogenate into 2,7ml reaction solution (30 % trichloroacetic acid, 0.75 % thiobarbituric acid, 5Nhydrochloric acid, 1:1:1, w/v) and heated at 100 0C for 15 min. The mixture was cooled to room temperature, centrifuged (3,000 g for 10 min), and the absorbance of the supernatant was recorded at 532 nm. 1,1,3,3-tetramethoxypropane was used as MDA standard. MDA results were expressed as μmol/mg wet tissue. The coefficients of intra-and inter-assay variation were 2.9 % (n=15) and 3.5 % (n=15), respectively.

Measurements of superoxide dismutase (Cu, Zn-SOD) activity: Cu, Zn-SOD activity was determined with the method of Sun et al. [13] by inhibition of nitroblue tetrazolium (NBT) reduction, with xanthine/xanthine oxidase used as a superoxide generator. One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50 %. The absorbance of each sample was read at 560 nm. SOD activity were expressed as U/mg wet tissue. The coefficients of intra-and inter-assay variation were 2.9 % (n=15) and 3.6 % (n=15), respectively.

Measurements of PCO levels: The kidney tissue PCO levels were determined by a commercially available enzyme-linked immunosorbent assay kit (BioAssay Technology Laboratory, E0870Ra, Shanghai, CHINA). PCO concentrations were expressed ng/mg wet tissue. The absorbances of the samples were measured by spectrophotometer at 540 nm. The coefficients of intra- and interassay variations were 4.3% (n=15) and 5.4% (n=15), respectively.

Measurements of AOPPs levels: Spectrophotometric determinations of AOPPs levels were performed using Witko-Tarsat et al. [14] method. The linear range of chloramine-T absorbance at 340 nm occurs between 0 and 100μmol/L. AOPP levels are expressed in μmol/mg of chloramine-T equivalents.

The coefficients of intra-and inter-assay variation were 2.8% (n=15) and 3.2% (n=15), respectively.

Light microscopy

Kidney tissue samples were fixed in 10% neutral formalin, followed by embedding in paraffin wax and then cut into 5-µm-thick sections. Periodic-Acid-Schiff (PAS) staining was performed.

Immunohistochemistry

Immunohistochemical analysis was performed using both Histostain-Plus Bulk Kits(85-043, Invitrogen) and Ultra Vision Antibody Detection System (LabVision), including mouse monoclonal bcl-2(Sc-7382, SCBT, 1:50 dilution), mouse monoclonal bax (Sc-7480, SCBT, 1:50 dilution) and rabbit polyclonal caspase-3(sc-7148, SCBT, 1:50 dilution) antibodies as described previously [3].

Semiquantitation of immunoperoxidase staining: Immunostaining was evaluated using a Leica DM2500 light microscope (X40 objective, Leica Microsystems, Wetzlar, Germany). Bcl-2, bax and caspase-3 immunostainings were analyzed and scored from 1+ to 3+ (1+refers to weak, while 3+to strong immunopositivity). This analysis was performed in a blind and randomized fashion of all stained sections.

TUNEL method (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

Detection of DNA fragmentation in situ was visualized with the use of the ApopTag Plus Peroxidase In Situ Apoptosis Detection Peroksidase Kit (S7101-KIT,Millipore), as described by the manufacturer. TUNEL assay was performed as described previously [3]. Staining was evaluated using a light microscope after counterstaining with methyl green.

Staining specificity controls

Thymus tissue sections from dexamethasone-treated rats were used as positive control. For negative controls, distilled water was used instead of Tdt enzyme.

Apoptotic index

Marked apoptotic cells were counted under a Leica DM2500 light microscope (X40 mag.). All TUNEL positive cells in randomly selected 12 different unit areas were counted on the cross-sections by a blinded researcher. Average cell per unit area number for each set of specimens in each group was calculated and compared.

Statistical analysis

The all datas of the 5 group of rats were compared using GraphPad Prism 5 software (San Diego, CA, USA) statistical package. The data were expressed as mean±SD. P value of less than 0.05 was considered statistically significant.

Results

Blood glucose level (Bg, mg/dL) and body weights (Bw, g)

At the beginning of the study, there was no statistically significant difference between the blood glucose levels of rats belonging to all groups (p=0.221). 72h after the induction of diabetes, Bg levels in untreated-diabetics were found to be significantly higher than the levels of the controls (p<0.001 for all). There was a significant increase in all diabetics compared to controls on the 15th day. At the end of the experimental period, Bg levels in untreated and quercetin-treated diabetics were higher than the controls (p<0.001 for all). Bg levels of quercetin-treated diabetics were significantly decreased when compared to untreated-diabetics (p=0.02) (Table 1). At the end of the experimental period, body weights of untreated-diabetic and quercetin-treated diabetics were significantly decreased when compared to controls (p=0.006, p=0.0002; respectively) (Table 1).

Microalbuminuria level (mg/L/24 h)

At the 1st and 15th day, there was a significant increase in microalbuminuria levels of either diabetic groups compared to control groups (p<0.01 for all). At the end of experiment, the microalbuminuria levels were lower in the quercetin-treated diabetics compared to the untreated-diabetics (p=0.003) (Table 1).

Daily urine volume (ml/day)

On days 15th and 30th, urine volume of diabetics showed a significant increase compared to controls (p<0.01 for all). At 30th days, a significant decrease was observed in the quercetin-treated diabetics compared to the untreated-diabetics (p=0.01) (Table 1).

Kidney weights (Kw, mg)

Kidney weights were significantly higher in the untreated-diabetics compared to the controls (p=0.006). In the quercetin-treated diabetics were significantly decreased when compared to untreated-diabetics (p=0.006) (Table 1).

MDA, SOD, AOPP and PCO levels

Mean kidney tissue MDA, AOPP and PCO levels of untreated-diabetics were significantly increased when compared with the controls (p=0.0002, p=0.0001, p=0.001; respectively) (Table 2). However; mean MDA, AOPP and PCO levels in the quercetin-treated diabetics were significantly decreased when compared with the untreated-diabetics (p=0.001, p=0.001, p=0.0005; respectively) (Table 2). Mean renal tissue SOD activities of untreated-diabetics were significantly decreased in comparison with the controls (p=0.0001). SOD activities of quercetin-treated diabetics were significantly increased in comparison with the untreated-diabetics (p=0.005) (Table 2).

Table 1: Blood glucose levels (Bg, mg/dL), body weight (Bw, g), Albuminuria level (Alb, mg/L/24 h), daily urine volume (ml/day), kidney weight (Kw, mg) and apoptotic cell count at the end of the experiment.

Groups (n=8)	Bg	Bw	Alb	Urine output	Kw	Apoptotic cell count
Control $^{\mu}$	107.7±4.9	354.1±23.7	0.01±0.008	11.3±3.0	1138±127.4	1.65±0.3
Quercetin $^{\mu}$	105.6±5.5	323.7±23.4	0.01±0.006	11±1.5	1150.7±107.1	1.71±0.3
Untreated-diabetic $^{\mu}$	572.7±26.7 ^{a}	303.7±33.5 ^{c}	0.15±0.004 ^{e}	66.6±7.5^{e}	1337.9±66.1 ^{h}	13.79±3.1^{i}
Diabetic + Quercetin $^{\mu}$	521±43.8 ^{a,b}	295.3±18.7 ^{d}	0.08±0.007^{e,f}	56.6±6.0 ^{e,g}	1155.1±122.3 ^{c}	6.41±1.7 ^{j}
p	^{a}p<0.001 ^{b}p=0.02	^{c}p=0.006 ^{d}p=0.0002	^{e}p <0.01 ^{f}p=0.003	^{g}p=0.01	^{h}p=0.006 ^{c}p=0.006	^{i}p = 0.0001 ^{j}p = 0.0001

$^{\mu}$: mean±SD, ^{a}p, ^{c}p, ^{d}p, ^{e}p, ^{h}p, ^{i}p versus control groups; ^{b}p, ^{f}p, ^{g}p, ^{j}p versus untreated diabetic group.

Table 2: Comparison of kidney tissue levels of MDA, SOD, PCO and AOPP in the four study groups.

Groups (n=8)	MDA[¥]	AOPP[¥]	PCO[β]	SOD[§]
Control[μ]	10.75 ±1.8	21.13±3.6	134.90±16.2	0.82±0.2
Quercetin[μ]	10.57±1.5	24.71±3.5	132.08±18.1	1.07±0.1
Untreated-diabetic[μ]	19.42±2.4[a]	120.77±20.3[c]	166.44±10.5[e]	0.22±0.1[c]
Diabetic + Quercetin[μ]	16.58±1.6[b]	65.85±13.3[d]	133.47±14.9[d]	0.61±0.3[f]
p	[a]p=0.0002 [b]p=0.001	[c]p=0.0001 [d]p=0.0005	[e]p=0.001 [d]p=0.0005	[c]p=0.0001 [f]p=0.005

[¥]: μmol/mg, tissue, [β]: ng/mg, tissue, [§]: U/mg, tissue, [μ]: mean±SD, MDA: malondialdehyde, AOPP: advanced oxidation protein products, PCO: protein carbonyl oxidation SOD: superoxide dismutase, [a]p, [c]p, [e]p versus control groups; [b]p, [d]p, [f]p versus untreated diabetic group.

Histopathological findings

Glomeruli and tubule structures were similar and uniform in controls (Figure 1A-B). In the untreated-diabetics showed tubular degeneration, mesangial matrix thickening in glomeruli and increased of mesangial cells (Figure 1C). In the quercetin-treated diabetics, the renal structural alterations were less distinct compared to those of untreated-diabetics (Figure 1D).

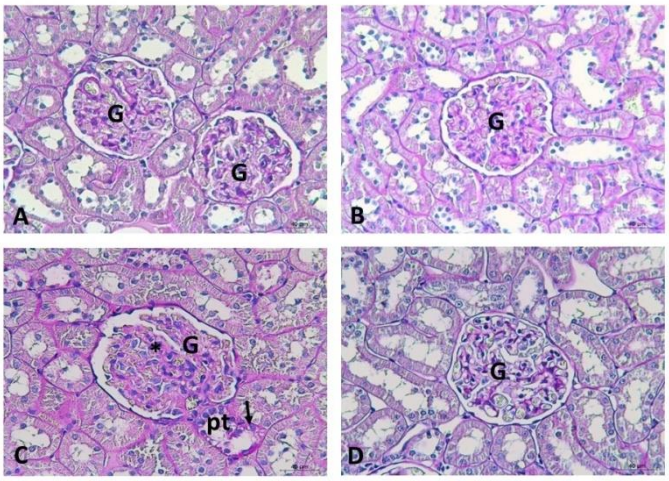

Figure 1. Light photomicrographs of PAS-stained sections of kidney sections. Non-diabetic (A) and quercetin-treated control groups (B). In the untreated-diabetic group showed tubular degeneration (↓), mesangial matrix thickening in glomeruli and increased of mesangial cells (*) (C). In the quercetin-treated diabetic group, the renal structural alterations were less distinct compared to those of untreated diabetic rats (D). (pt: proximal tubuli; G:glomeruli), Bar: 40μm.

Immunohistochemical findings

Bcl-2 and bax

The immunopositiviy of bcl-2 (Figure 2A) and bax (Figure 3A) was weakly observed in the tubules and glomeruli of controls. In untreated-diabetics, bcl-2 (Figure 2C) and bax (Figure 3C) immunopositivity were more prominent in the tubules and glomeruli comparison with that of the controls. In quercetin-treated diabetics, bcl-2 (Figure 2D) and bax (Figure 3D) intensity were weaker than that of the untreated-diabetics.

Caspase-3

Very few caspase-3 immunopositive cells were detected in the glomeruli and tubules in the controls (Figure 4A-B). Immunostaining of caspase-3 was markedly increased in glomeruli and damaged tubuli of the untreated-diabetics (Figure 4C). A significant decrease in the numbers of caspase-3 immunopositive glomeruli was observed in the quercetin-treated diabetics compared to untreated-diabetics (Figure 4D).

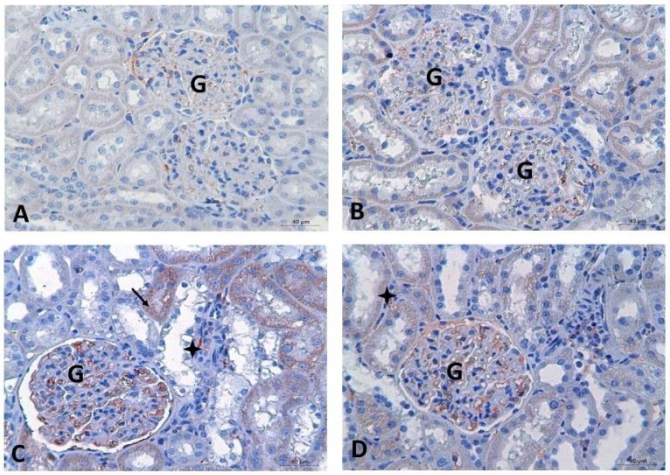

Figure 2. Immunoreactivity of bcl-2. The immunopositiviy of bcl-2 was weakly observed in the tubules and glomeruli of non-diabetic (A) and quercetin-treated (B) control groups. In untreated-diabetic group, bcl-2 immunopositivity were more prominent in the tubules and glomeruli (C). The quercetin-treated diabetic group, showed decreased bcl-2 immunoreactivity in the all sections compared to untreated-diabetic rats (D)(G:glomeruli; ↑:immunopositivity; ✦:interstitial area). Counterstain: Hematoxylin, Bar:40μm.

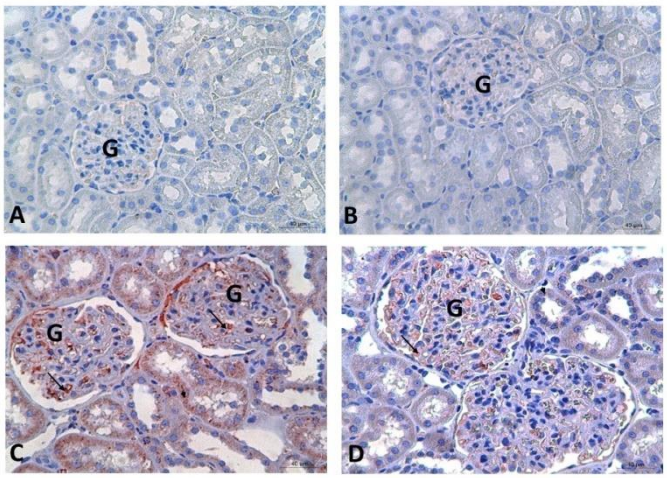

Figure 3: Immunoreactivity of Bax: Non-diabetic (A) and quercetin-treated (B) control groups. Intense cytoplasmic immunostaining of Bax was observed in the glomeruli (↓) (B) and at distal tubuli of untreated STZ-diabetic group (►) (C). In the quercetin-treated diabetic group, weak bax immunoreactivity was observed in the glomeruli (D)(G:glomeruli; ↓:immunopositivity). Counterstain: Hematoxylin, Bar:40μm.

TUNEL method

After staining with TUNEL method, cells with brown staining of nuclei were evaluated as apoptotic. The positive control tissue section (Figure 5A). In the controls, very few apoptotic cells were observed in the cortex, medulla and distal tubuli but no staining was observed in the glomeruli (Figure 5B). However, the number of apoptotic cells increased especially in the medullary area and distal tubuli of the untreated-diabetics compared to the controls (p=0.0001), whereas apoptosis was detected in the cells of the some injured glomeruli (Figure 5C) (Table.1). A significant decrease was also observed in the quercetin-treated diabetics (p=0.0001) compared to untreated-diabetics (Table 1) (Figure 5D).

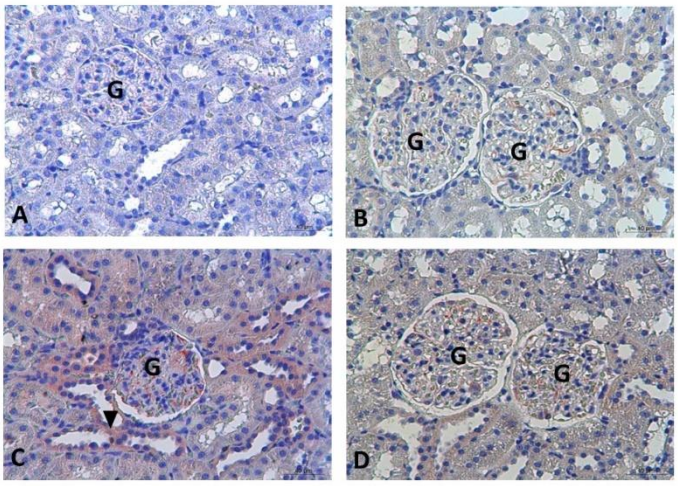

Figure 4. Immunoreactivity caspase-3. The caspase-3 immunostaining was increased in damaged glomeruli and tubuli of untreated diabetic group (thick arrow) (C) compared to controls group (A, B). In the quercetin-treated diabetic group, a significant decrease of caspase-3 immunoreactivity was observed (D). (G: glomeruli;►: immunopositivity). Counterstain: Hematoxylin, Bar: 40µm.

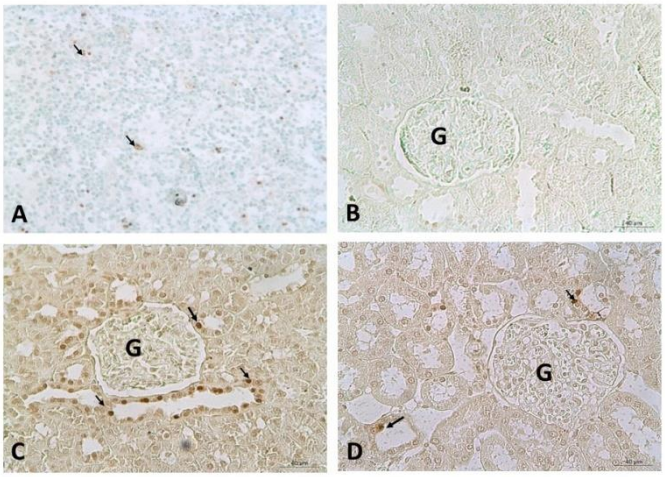

Figure 5. The kidney tissue samples stained with TUNEL method. Arrows refer to apoptotic cell nucleus. Positive control tissue section (A). Control groups demonstrated very few apoptotic cells were in cortex (B). Untreated-diabetic group group showed more apoptotic cells than other groups (C). Quercetin-treated diabetic group showing less few apoptotic cells(D).(G:glomeruli; ↓: apoptotic cell nucleus) Bar:40µm, Counterstain: Methyl green.

Discussion

Diabetes mellitus is a serious chronic and metabolic disease that is common in all countries, leading to impaired carbohydrate, protein and fat metabolism resulting in either insufficient insulin action or loss of insulin action in target tissues [11, 15]. The most common microvascular complication of DM is diabetic nephropathy. Hemodynamic, metabolic and genetic factors as well as oxidative stress play an important role in the pathogenesis of DN [16].

In studies, STZ, a diabetic agent, is administered intraperitoneally in a single dose of 40-60mg/kg due to its specific acute toxicity to pancreatic β-cells to produce experimental diabetes [3, 11]. After STZ injection, the presence or absence of diabetes is determined by observing the blood glucose levels (>250mg/dl) within 2-3 days [3]. Since diabetes is an oxidative stress disorder, antioxidant administration in diabetic animal models has been shown to reduce hyperglycemia caused by STZ. In our study, rats were observed hyperglycemic according to the results of blood glucose measurements performed 72-hours after STZ injection and hyperglycemia was observed during the study. Consistent with studies reporting that antioxidant administration reduced blood glucose level [11], a significant decrease observed in the quercetin-diabetics.

The earliest diagnosis of diabetic nephropathy is possible with the appearance of abnormal microalbuminuria in the urine. Experimental studies have shown that the elevation of the blood glucose is effective at the onset and progression of microalbuminuria [3]. In our study, microalbuminuria results were found to be consistent with expected microalbuminuria levels in diabetic nephropathy model. In the quercetin-treated diabetics were observed decrease in microalbuminuria values with compared the untreated-diabetics. It was assessed in agreement with the decrease in blood glucose levels.

Short and long-term experimental diabetes studies have reported a significant decrease in body weight levels in diabetic rats [11, 17]. In our study, the decrease in body weight of diabetics were found to be consistent with the results of other studies. In addition, some studies reported that antioxidant treatment prevented weight loss in diabetic rats [18, 19]. In the present study, quercetin-treated diabetics displayed less body weight, loss than the untreated-diabetics. This suggests that quercetin may help the maintenance of the body weight by controlling blood glucose level.

There are conflicting research results about the diabetes ans its effect on kidney weight. Tunçdemir et al.[3] reported that diabetic rats showed significantly increased kidney weights due to hypertrophy compared with controls, whereas Elbe et al.[11] reported no difference in kidney weights of diabetics compared to the other groups. In our study, kidney weights of diabetic rats were significantly increased compared to those of controls, and in the quercetin-treated diabetics was significantly decreased compared to untreated-diabetics.

Many studies have reported that hyperglycemia leads to oxidative stress by increased free oxygen radicals and reducing antioxidant capacity, and adverse effects on the kidney, such as in other diabetic complications [11, 18]. Antioxidant enzymes such as SOD separates superoxide and hydrogen peroxide to its components in the cell [20]. It has been reported that SOD enzyme activity is decreased and the MDA level is increased in many tissues of diabetic rats that antioxidant administration increases tissue SOD level and decreases MDA level [20, 21, 22]. Obrosova et al. [23] reported that MDA levels in the renal cortex as well as SOD activity were increased early in diabetes. Dias et al. [22] reported an increase in SOD activity in their diabetic liver studies. We think that this difference may be due to tissue specificity and duration of the disease. In our study, renal tissue SOD enzyme activity in the diabetic group was found to be decreased compared to that in control groups while MDA levels were found to be increased. It has also been suggested that

the SOD levels of quercetin-induced diabetics are increased when compared to the untreated-diabetics and the MDA levels are decreased.

The increased oxidative stress leads to the formation of protein carbonyl derivatives and AOPPs for protein oxidation by reducing the natural antioxidant capacity of the body [24]. In diabetic pancreas, liver and serum, AOPP and PC-related studies [25, 26] as well as there is a study on diabetic kidney tissue with these parameters [27]. Shi et al. [27] reported that the renal tissue AOPP and PC levels of the diabetic group were significantly increased compared to the control group. Quercetin is a powerful antioxidant with the ability to free radical scavenging and inhibit superoxide radicals via xanthine oxidase [20]. Many studies report that oxidative stress due to diabetes is inhibited by quercetin [11, 20]. In our study, AOPP and PC values were found higher in the diabetics compared to the controls, whereas in the quercetin-treated diabetics, it was decreased compared to the untreated-diabetics. Considering the oxidative stress parameters, we can say that quercetin administration significantly reduces oxidative stress.

Histopathological damage is seen everywhere in all over the diabetic kidney [3, 11]. We detected some diabetes-related alterations including mesangial cell enlargement and matrix thickening in the glomerule in addition to tubular basement membrane thickening with degenerations. It was observed that injury observed in glomeruli and tubules in the quercetin-treated diabetics were reduced compared to the untreated-diabetics.

It has also been reported that apoptosis plays a role in the development of oxidative stress as well as in the development of renal damage [6, 28]. Studies on how quercetin affects apoptosis have been conducted with cancer cell lines such as hepatoma, glioblastoma, and osteosarcoma, and results have proven that quercetin induces apoptosis [29, 30]. Kanter et al.[19] reported that the anti-apoptotic effect of quercetin in the diabetic testis. However, we did not find a comprehensive study of the effects of quercetin on apoptosis in the model of diabetic nephropathy. In our study, in the untreated-diabetics, bcl-2 (anti-apoptotic) immunopositivity increased especially in the glomeruli and occasionally in the tubules compared to the controls. Bax (pro-apoptotic) and caspase-3 immunopositivity was found to be associated with an increase in the number of apoptotic cells detected in the renal tissue of diabetics. In the quercetin-treated diabetics, immunopositivity of bcl-2, bax and caspase-3 were significantly reduced compared to the untreated-diabetics, and a significant decrease in the number of apoptotic cells parallel to these findings was found.

In conclusion, in our experimental diabetes model, we observed that quercetin over the dose and duration of use significantly improved blood glucose, microalbuminuria, renal weight, daily urinary excretion and loss of body weight. As a result of histopathological evaluations, we observed that the diabetes specific characteristic morphological changes in the kidney tissues of the untreated-diabetics and the increase in bcl-2, bax, caspase-3 immunopositivity decreased with quercetin administration. We found that the increase in the number of apoptotic cells in the tissue sections of the diabetic group was significantly reduced by quercetin administration. In addition, we have reported that quercetin-treatment increases the activity of MDA, the lipid peroxidation product, the activity of antioxidant enzyme, SOD, in contrast attenuates the oxidative stress markers AOPP and PCO compared with the untreated-diabetics.

In experimental diabetic nephropathy model, the flavonoid quercetin, may have protective effects against damages caused by diabetes in kidney tissue during the applied dose and time, may act on apoptotic regulatory proteins to protect the cells from apoptosis by increasing the level of antioxidants (Figure 6).

We believe that the antiapoptotic effects of quercetin, which is predominantly antioxidant, may be useful in reducing the effects of diabetic complications and preventing new complications. We suggest that the effects on different diabetic complications at different doses and durations can be extensively investigated in future studies.

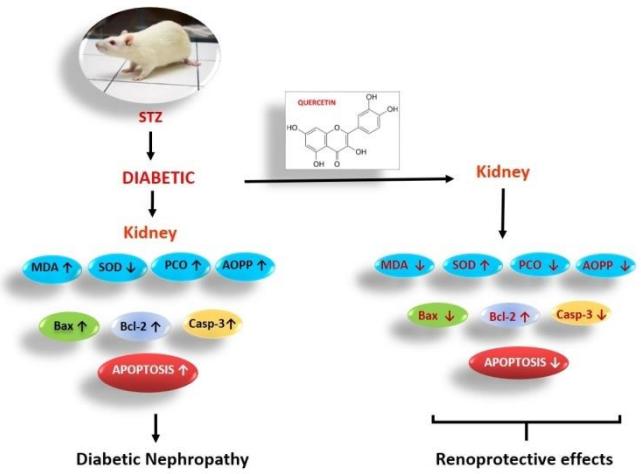

Figure 6. Protective effects of quercetin on diabetic nephropathy in rats. The quercetin may have protective effects against damages caused by diabetes in kidney tissue, may act on apoptotic regulatory proteins to protect the cells from apoptosis by increasing the level of antioxidants in experimental diabetic nephropathy model.

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Arch Clin Exp Med 2018;3(3):186-189. e-ISSN: 2564-6567

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Biliary fistula after liver hydatid cyst surgery: Is it a predictable complication?

Karaciğer hidatik kist cerrahisi sonrası safra fistülü: Tahmin edilebilir bir komplikasyon mu?

Önder Karabay [1], Özgür Bostancı [2]

Abstract

Aim: The biliary fistula development after liver hydatid cyst surgery is a common complication. The aim of this study is to examine the factors affecting the development of postoperative biliary fistula in patients operated for liver hydatid disease.

Methods: The study was retrospectively performed in patients treated surgically for liver hydatid cyst between 1999 and 2010. The data of 53 patients operated for hydatid csyt were reviewed with hospital records. Patients were divided into two groups as biliary fistulas with (Group A) and without biliary fistula (Group B). The demographic data (age, sex), cyst diameter, cyst localization, laboratory tests and length of hospital stay were recorded. These parameters were compared with the groups.

Results: The groups were similar in terms of age and sex (p = 0.790 and p=1.0, respectively). In group A, the mean cyst diameter was significantly higher than group B (p=0.001). The mean duration of hospitalization was longer in group A than group B (p=0.001). There was no difference between the groups considering cyst localization, AST, ALT, total bilirubin and direct bilirubin (p>0.05 for all).

Conclusion: Preoperative cyst diameter may be a valuable parameter for predicting biliary fistula preoperatively. However, larger prospective studies are needed on this subject.

Key words: Hydatid cyst, liver, biliary fistula, ERCP

Öz

Amaç: Karaciğer hidatik kisti ameliyatı sonrası safra fistülü gelişimi sık bir komplikasyondur. Bu çalışmanın amacı, karaciğer hidatik hastalığı nedeniyle opere edilen hastalarda postoperatif bilyer fistül gelişimini etkileyen faktörleri incelemektir.

Yöntemler: Çalışma, 1999 ve 2010 yılları arasında karaciğer hidatik kisti nedeniyle cerrahi olarak tedavi edilen hastalarda retrospektif olarak yapıldı. Hidatik kist için ameliyat edilen 53 hastanın verileri hastane kayıtları ile gözden geçirildi. Hastalar safra fistülü olan (Grup A) ve safra fistülü olmayan (Grup B) olmak üzere iki gruba ayrıldı. Yaş, cinsiyet, kist çapı, kist yerleşimi, laboratuvar testleri ve hastanede kalış süresi kaydedildi. Bu parametreler gruplar arasında karşılaştırıldı.

Bulgular: Gruplar yaş ve cinsiyet açısından benzer özellikteydi (p = 0,790 ve p=1,0 sırasıyla). Grup A'daki ortalama kist çapı grup B'den anlamlı olarak büyüktü (p=0,001). Ortalama hastanede kalış süresi grup A'da grup B'den uzundu (p=0,001). Kist lokalizasyonu ve AST, ALT, total bilirubin, direk bilirubin değerleri açısından gruplar arasında fark yoktu (hepsi için p>0.05).

Sonuç: Ameliyat öncesi kist çapı, safra fistülünü tahmin etmek için faydalı bir parametre olabilir. Bununla beraber, bu konuda daha geniş prospektif serilere ihtiyaç vardır.

Anahtar Kelimeler: Kist hidatik, karaciğer, safra fistülü, ERCP

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Introduction

Liver hydatid disease is a common zoonotic disease in Turkey and the Mediterranean countries [1]. If it is not treated, it can lead to serious health problems. Although there are many innovations in treatment, surgical procedures continue to be widespread.

No matter how precisely surgical treatment is done, development of postoperative biliary fistula is still one of the most important complications. The causes of biliary fistulae are an association between the cyst and the biliary tract, an injury to the biliary tract, or a high intrabiliary system pressure due to the presence of scolexes in the main bile duct. In order to prevent this, magnetic resonance cholangiopancreatography (MRCP) scanning should be performed in suspicious cases before surgical treatment and especially in the presence of large cysts. If postoperative biliary fistulas persist for a long time or the drain output is high, it can rarely result in mortality. In appropriate patients, fistulas can be controlled by endoscopic procedures or percutaneous drainage [2]. Additionally, there isn't still a gold standard blood test for prevention of biliary fistula after liver hydatid cyst surgery.

The aim of this study is to examine the factors affecting the development of postoperative biliary fistula in patients operated for liver hydatid disease.

Material and methods

This retrospective study was performed in Sisli Etfal Education and Research Hospital in Istanbul, Turkey. Institutional review board approval was obtained. Informed consent could not be obtained from all participants for being included in this retrospective study. All of the procedures were in accordance with the World Medical Association Helsinki Declaration of 1964 and later versions.

Patients operated for liver hydatid cyst between January 1999 and December 2010, were evaluated retrospectively. There were a total of 64 patients. Eight patients with lack of clinical data and three patients with lack of follow up data were excluded. Therefore, 53 patients were included in the study.

Patients were analyzed for age, gender, laboratory values, imaging findings with regard to number, diameter, and location of the cysts, operation type, and length of hospital stay.

In the preoperative diagnosis, the patient's anamnesis, physical examination findings, and abdominal USG were used. All patients underwent a pre-operative CT scan. The location of the cyst in the liver, its size, number and its relation with surrounding tissues were examined. Laboratory analysis including alanine transaminase (ALT) (U/l), aspartate transaminase (AST) (U/l), total and direct bilirubin (mg/dl), were recorded in the preoperative period.

In the preoperative period, 10 mg/kg albendazole (Andazol, 200 mg, tablet, Biofarma, Turkey) was given for 3 weeks when the liver enzymes were normal. All the patients were informed about the surgery to be performed and the possible complications, and an informed consent form was obtained. Open or laparoscopic cystotomy and drainage were performed for all patients.

Surgical technique

Right subcostal incision was preferred in open surgical technique. Four trocars were used in the laparoscopic method. According to the placement of the cysts, trocar locations were regulated. The cyst was aspirated to reduce the pressure inside the cyst. A 0.09% NaCl solution was added into the cyst. After waiting for five minutes, the cyst was opened over, the germinative membrane and the female vesicles were removed from the cyst. The cyst was washed with saline and aspirated. The presence of bile in the cyst was assessed. If there was an open bile duct in the cyst, it was sutured. The drain was inserted into the cyst in all patients. The oral regime started the same day after surgery. In the postoperative period, 10 mg/kg albendazole was started again. Albendazole treatment was planned for three weeks usage and one week rest for six months period.

In the postoperative period, the presence of bile fluid in the drain tube was evaluated as a biliary fistula. Patients were evaluated in two groups: postoperative biliary fistula (Group A) and no fistula group (Group B).

The drain outputs were followed in patients with post-operative biliary fistula (Group A). If the drain output is low and biliary fistula is closed spontaneously, no intervention was made.

If there was a long-standing bile fistula on day 5 or a high-output biliary fistula (>500 ml), ERCP was performed. Nasobiliary drainage or sphincterotomy was performed during ERCP. The patients were followed up for 1 year after discharge. At 1st year follow-up, CT scan was performed.

Statistical analysis

Statistical analysis was performed using SPSS ver. 20 (SPSS, Chicago, IL, USA). All continuous data were presented as means ± standard deviations. Statistical significance of the findings was analyzed using the two-tailed Student's t-test, Pearson chi-square test, and Mann-Whitney test. A p-value less than 0.05 was considered to be statistically significant.

Results

Fifty-three patients who underwent hepatic hydatid cyst surgery were retrospectively reviewed. Twenty-nine of the patients were male (55%) and 24 were female (45%). Mean symptom duration was 10.7 months (range 1-120 months).

There were multiple cysts in 12 patients (23%) and single cysts in 41 patients (77%). The mean cyst size was 81.75 mm (range 30-140 mm). When we analyzed the cyst localization, in five patients (9.44%) were in both lobes, in 30 patients (56.60%) were in the right lobe and in 18 (33.96%) patients were in the left lobe.

Three patients (5.6%) were operated by the laparoscopic method and 50 patients (94.4%) were operated by the open technique. The mean duration of hospitalization was 6.6 days (2-36) days.

14 patients (26.41%) had biliary fistula after operation (Group A). The biliary fistula was seen in 13 patients (24.52%) who underwent open surgery and one case (33.3%) under laparoscopic surgery. Solitary cysts (85.72%) were present in 12 of the patients in Group A, and multiple cysts were present in two (14.28%).

The groups were similar in terms of age and sex ($p = 0.790$ and $p=1.0$, respectively). The mean cyst diameter was 101.07±23.4 mm in group A and it was significantly higher than group B ($p=0.001$). The mean duration of hospitalization was 11.79±10.6 (4-36) days in group A and it was significantly longer than group B ($p=0.001$). Comparison of the groups for cyst localization revealed no significant difference ($p=0.422$).

There was no difference between the groups considering AST, ALT, total bilirubin and direct bilirubin ($p>0.05$ for all) (Table 1).

In 14 patients (Group A), the mean daily drainage was 341.07±284 ml. Seven patients in Group A (50%) underwent ERCP (nasobiliary stent in two) and the remaining seven patients (50%) were followed up. The mean daily fistula drainage was 500 ml (range 200-950 ml) and 175 ml (range 75-350 ml), respectively. The mean drainage of two patients with nasobiliary stents was 800 ml (range 650-950 ml) (Table 2).

The median length of fistula closure was 11 days (range 6-60 days). In the group of patients who were followed up with fistulas, the median length of close time was 10 days (range 6-21 days). The median closure time of patients who was performed ERCP was 12 days (range 7-60 days). The mean follow up time of patients was one year and none of the patients had biliary fistula recurrence.

	Overall (n=53)	Group A (n=14)[γ]	Group B (n=39)[γ]	p
Cyst diameter (mm)[μ]	81.7 (30-140)	101.07±23.4	74.82±24.9	0.001
Localization				
Right	30	10	20	
Left	18	3	15	0.420
Bilobular	5	1	4	
AST (U/L)	57.13	32.14± 16.3	66.1± 80.8	0.290
ALT (U/L)	52.43	38.2±23.3	57.51±75.2	0.940
Total bilirubin (mg/dl)	0.85	0.65±0.37	0.92±1.1	0.311
Direct bilirubin (mg/dl)	0.33	0.21±0.26	0.37±0.48	0.030
Hospitalization time (day)[μ]	6.6 (2-36)	11.79±10.6	4.87± 1.5	0.001

Table 1. Comparison of preoperative features of the groups.
[μ]: mean (min-max), [γ]:mean±standard deviation

		Number (n)	Drainage (ml)[μ]
Biliary fistula	No	39	-
	Yes	14	341.07 (75-950)
Treatment	Conservative	7	175 (75-350)
	ERCP	7	500 (200-950)
	Nasobiliary stenting	2	800 (650-950)

Table 2. The drainage output of the patients with fistula.

[μ]: mean (min-max)

Discussion

Liver hydatid cyst disease is an important parasitic disease in terms of human and animal health. As it is nowadays in the world, it continues to be a serious health problem in our country as well. Many modalities were tried like medical treatment or percutaneous drainage with USG for hydatid disease. However, surgical treatments still maintain the efficacy and necessity. Biliary fistulas and recurrences are the most common complications after liver hydatid cyst surgery [2]. Factors that may be effective in the development of biliary fistulas; the high pressure in the cyst, a large relationship between the cyst and biliary system, an iatrogenic injury at the biliary system, ductus choledochus obstruction by germinative membranes and scolexes. It is stated that 80-90% of the small cystobiliary connections are present but clinically the connection is seen in 13-37% [3, 4]. The cystobiliary connection was presented with the appearance of bile leakage into the cyst during surgery or by imaging the connection in the ERCP due to jaundice or biliary fistula in the postoperative period [3].

In literature, there were many different biliary fistula rates. In a study with 117 patients, Patkowski et al reported biliary fistula in three patient (2.5%) [5]. Furthermore, Nooghabi et al reported 27.3% biliary fistula rate in 73 patients [6]. In large number of studies; Baraket et al reported 8 biliary fistulas in 120 patients (10.4%), Surmelioglu et al reported 36 fistulas in 186 patients (19.4%) and Nakeeb et al reported 13 fistulas in 123 patients (10.5%) [7, 8, 9]. In our study, we found postoperative biliary fistula in 14 patients (26.4%). The rate of biliary fistula in our study is higher than literature. The low number of patients and the presence of large diameter cysts may be the reason for the high fistula rate in our study.

There is not a gold standard surgery technique in liver hydatid disease for decreasing the biliary fistula rate. So, the biliary fistula is still a common complication after surgery. Although, there are many ideas to prevent the fistula and none of them are effective at 100%. In literature, there were a few studies which researched on the parameters to prevent the biliary fistula [1, 2]. The study of Kilic et al, liver enzymes (ALT, AST, GGT, ALP) and cyst type-diameter were evaluated and the difference was statistically significant about only cyst diameter [1]. Atahan et al reported that GGT can be a predictive test for preventing the biliary fistula. Furthermore, they didn't find a difference for cyst diameter in groups [2]. Liver enzymes and cyst features were examined in this study and only cyst diameter was significantly associated with biliary fistula.

Patients with biliary fistulas are also more likely to stay in the hospital than other patients. In a study conducted by Agarwal et al. [10], the mean duration of hospital stay in patients with fistulas was 18 days, while the mean duration of fistula-free patients was seven days. In our study, the mean hospital stay of biliary fistula group was significantly longer than fistula-free group.

There are several modalities in the treatment of biliary fistulas. It was observed that some of the biliary fistulas were spontaneously closed during follow-up. Balik et al reported 10 biliary fistulas in 304 patients and they were spontaneously closed during the follow-up period of 2-4 months [11]. In the study of Vagioanos et al, 7 of 12 biliary fistulas were spontaneously closed in 38 days [12]. In our study, 7 of 14 biliary fistulas were spontaneously closed in follow up. The others needed endoscopic intervention. Although some biliary fistulas can be closed spontaneously, there may be persistent fistulas. Most of the fistulas don't require re-surgery and can be treated by endoscopic or percutaneous interventions [10]. Today, endoscopic interventions are the mainstay of treatment in patients with unclosed or high-flow fistulas. It is believed that endoscopic sphincterotomy causes the early closing of the fistula by lowering intrabiliary pressure [13]. There is no definite conclusion about the timing of the endoscopy. There are different opinions between the next few days and a few months [14, 15]. In our study, there were not required a second surgery in patients with fistulas. Seven patients who have persistent fistulas were treated with endoscopic sphincterotomy (five patients) or nasobiliary stenting (two patients). All of the patients with biliary fistula were treated successfully.

Limitations of this study are the small number of patients and tests, and to be retrospective. In a prospective study with high volume series, it may be more effective and guiding.

In conclusion, preoperative cyst diameter may be a valuable parameter for predicting biliary fistula preoperatively. However, larger prospective studies are needed on this subject.

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Effects of α-lipoic acid on oxidative stress parameters in experimental hyperthyroidism

Deneysel hipertiroidizmde α-lipoik asidin oksidatif stres parametreleri üzerine etkileri

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Abstract

Aim: To investigate the effects of α-lipoic acid (ALA) on prooxidant-antioxidant balance in liver tissue, as well as liver function tests in experimental hyperthyroidism.

Materials and Methods: For the evaluation of prooxidant-antioxidant balance, reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl (PC), ferric reducing antioxidant power (FRAP), glutathione (GSH) levels, and superoxide dismutase, catalase and glutathione peroxidase activities were determined. Histopathological examinations were also performed. Hyperthyroidism was induced by the administration of L-thyroxine [T4, 12 mg/L] in drinking water for 10 weeks. The ALA [100 mg/kg/day; 0.2% (w/w) in diet] was administered in last 5 weeks of experimental period.

Results: Oxidative stress in liver tissue from hyperthyroid rats was accentuated. Significant increases in hepatic ROS, MDA, and PC levels were found. Additionally, increased FRAP and decreased GSH levels were observed. ALA treatment lowered the elevated serum free T3 and T4 levels and significantly decreased hepatic ROS, MDA and PC levels. Serum liver function tests in hiperthyroid rats before and after ALA treatment were not changed.

Conclusion: Our results indicate that ALA treatment was effective in the improvement of changes in prooxidant-antioxidant balance, and may be useful as supportive agent for the treatment of hypertyroidism.

Key words: Proxidant-antioxidant balance, hyperthyroidism, α-lipoic acid.

Öz

Amaç: Deneysel hipertiroidizmde α-lipoik asid (ALA)' in karaciğer dokusunda prooksidan-antioksidan denge üzerine etkileri ile karaciğer fonksiyon testlerinin incelenmesi.

Yöntem: Prooksidan-antioksidan dengenin değerlendirilmesi için, reaktif oksijen ürünleri (ROS), malondialdehit (MDA), protein karbonil (PC), total antioksidan kapasite (FRAP) ve glutatyon (GSH) düzeyleri ile süperoksit dismutaz, katalaz ve glutatyon peroksidaz aktiviteleri incelendi. Ayrıca, histopatolojik incelemeler yapıldı. Hipertiroidi tablosu oluşturmak için T4 (12 mg/L) 10 hafta boyunca içme suyunda uygulandı. ALA [100 mg/kg/gün; % 0.2 [w/w]; diyette] deney süresinin son 5 haftasında uygulandı.

Bulgular: Karaciğerde oksidatif stresin arttığı görüldü. Hipertiroidili sıçanlarda ROS, MDA, PK düzeylerinde anlamlı artış bulundu. Ayrıca FRAP düzeylerinde artış ve GSH düzeylerinde azalma görüldü. ALA tedavisi, artan serum serbest T3 ve T4 düzeylerini düşürdü ve karaciğerde ROS, MDA ve PK düzeylerini anlamlı olarak azalmasına neden oldu. ALA uygulaması öncesi ve sonrasında serum karaciğer fonksiyon testlerinde bir değişiklik görülmedi.

Sonuç: Sonuçlarımız, ALA tedavisinin prooksidan-antioksidan dengedeki değişikliklerin düzelmesinde etkili olduğunu ve hipertiroidi tedavisinde destekleyici ajan olarak yararlı olabileceğini göstermektedir.

Anahtar Kelimeler: prooksidan-antioksidan denge, hipertiroidizm, α-lipoik asid.

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Introduction

It is well known that a delicate balance exists between the rates of reactive oxygen species ([ROS) formation and their neutralisation. Protection of body against disturbances in prooxidant-antioxidant balance, is a multi-factorial process involving many cellular metabolic pathways. Increased ROS formation affects macromolecules such as lipids, proteins, DNA, and may alter their structure and function, resulting in cell damage [1]. Thyroid hormones (thyroxine and triiodothyronine, T3, T4) are necessary for the various physiological processes such as growth, differentiation, development and reproduction [1, 2].They are also necessary for the regulation of lipid and carbohydrate metabolism, and oxygen utility. In addition, thyroid hormones are impilicated in ROS formation in the cell by altering basal metabolism and respiratory chain reactions [2-4]. On the other hand, decreasing the availability of non-enzymatic antioxidants or increasing the expression of certain antioxidant enzymes thyroid hormones affect antioxidant mechanisms, thus alter prooxidant-antioxidant balance [5, 6]. Indeed, many clinical and experimental studies [7, 8] showed that oxidative stress develops in hyperthyroidism, and that the susceptibility to oxidative stress was different among tissues. Because of its rich mitochondrial content, and due to the important role in body metabolism and drug detoxification, the liver is among the most frequently studied tissues for evaluation of prooxidant-antioxidant balance.

Recently, it has been suggested that antioxidant therapy may be helpful for preventing the oxidative stress seen in hyperthyroidism and may provide support to the classical antithyroid treatment. For this purpose, various antioxidants such as vitamin E, curcumin, and quercetin have been used in hyperthyroidism and some favorable results have been obtained [7, 9-11]. α-Lipoic acid (ALA) is a mitochondrial coenzyme with significant antioxidant properties. ALA supports regeneration of many antioxidants such as glutathione (GSH), coenzyme Q10, vitamin E and C, repairs oxidized proteins, creates complexes with metal ions such as copper, manganese, and zinc, and prevents formation of ROS [12]. Because of these properties, it has been reported that ALA possess beneficial effects in various conditions related with accentuated oxidative stress [12-15]. To the best of our knowledge, the effects of of ALA in hyperthyroidism was not been investigated yet. Therefore, the aim of our study was to examine changes in prooxidant-antioxidant balance in liver tissue obtained from hyperthyroid rats, and to evaluate the effects of ALA on oxidative stress parameters, as well as liver function tests in experimental hyperthyroidism.

Material and methods

The investigation was performed on male Sprague-Dawley albino rats (weighing 250-350 g) purchased from Aziz Sancar Institute for Experimental Medical Research, Istanbul University, Turkey. Animals were housed in a light- and temperature-controlled room on a 12/12 hours light/dark cycle. All experiments met the guidelines the Animal Care and Use Committee of Istanbul University [Project No. 2014/111]. All chemicals were supplied from Sigma-Aldrich [St. Louis, MO, USA].

Rats were randomly divided into four groups (6 rats per group) as control, ALA, T4 and T4+ALA. Rats of the control group were fed with standard diet ad libitum for 10 weeks. Rats in ALA group were fed initially with standard diet for 5 weeks. For 5 weeks following period, rats were fed with ALA [100 mg/kg/day; 0.2% (w/w)] supplemented diet. Tap water was given as drinking water in control and ALA groups. Hyperthyroidism (T4 and T4+ALA groups) was induced by administration of L-Thyroxine sodium pentahydrate (T4) in drinking water to a final concentration of 12 mg/L for 5 weeks. Three weeks after the administration of T4, free T3 (fT3) and free T4 (fT4) levels were determined in rats randomly chosen from the study groups. After completion of 5 weeks period, administration of T4 was continued for another 5 weeks (T4 and T4+ALA groups). Rats of T4+ALA group were fed with 0.2% (w/w) ALA containing diet for last 5 weeks.

At the end of study period (10 weeks), after 10-12 hours fasting period blood samples were obtained by cardiac puncture under pentobarbital anesthesia (50 mg/kg, i.p.) in plain tubes. Blood samples were centrifuged at 1500 × g (10 min, 4□C) to remove the serum. Liver tissue were removed immediately after blood collection, rinsed with ice-cold saline, blotted with filter paper. Liver tissue homogenizate (10%; w/v) was prepared in ice-cold 0.15 M KCl. Homogenates were centrifuged at 600 × g min to obtain supernatants where reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl (PC), ferric reducing antioxidant power (FRAP), glutathione (GSH) and catalase (CAT) activity were measured. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined in postmitochondrial fraction obtained after cantifugation at 10,000 × g for 20 min of supernatants.

Determinations in serum

Serum fT3 and fT4 measurements were performed on a Elecsys automated analyzer (Roche Diagnostics, Mannheim, Germany). Serum alanine aminotransferase [ALT] and aspartate aminotransferase [AST] activities were determined by Cobas Integra 800 automated analyzer (Roche Diagnostics, Mannheim, Germany).

Determinations of ROS formation, lipid peroxidation and protein oxidation

ROS formation was measured fluorometrically [16]. After incubation with 2,7-dichlorodihydrofluorescein diacetate [DCFH-DA], the fluorescence of 2,7-dichlorofluorescein was read on a microplate fluorometer (Fluoroskan Ascent FL, Thermo Scientific Inc, USA)(excitation of 485 nm and emission of 538 nm). Lipid peroxidation was determined by measuring the levels of MDA by thiobarbituric acid test [17]. The absorbance was read at 535 nm against the blank. Molar extinction coefficient of 1.56×105 M-1.cm-1 was used for calculation of MDA levels. As a marker of protein oxidation PC levels were determined by the measuring of carbonyl groups reacting with 2,4-dinitrophenylhydrazine (DNPH)[18]. Molar absorbtion coefficient of 22,000 M−1 cm−1 at maximum absorbance (360 nm) was used for calculation of PC levels. For the spectrophotometrical measurements Ultraspec 3000 spectrophotometer (Pharmacia Biotech, Biochrom Ltd. Cambridge, UK) was used.

Determinations of non-enzymatic and enzymatic antioxidants

Ferric reducing antioxidant power (FRAP), reflecting total antioxidant status, was measured according to Benzie and Strain method [19]. Glutathione (GSH) levels were measured with 5,5-dithiobis-2-nitrobenzoate at 412 nm [20]. For catalase (CAT) activity measutement as a substrate hydrogen peroxide was used [21]. SOD activity measutement was based the effect of riboflavin-sensitized photooxidation of o-dianisidine [22]. For GSH-Px activity [23] measurement cumene hydroperoxide was used as substrate. Protein levels were determined using bicinchoninic acid [24].

Histopathologic evaluation

Liver tissues were fixed in 10% buffered formaldehyde, processed, and stained with hematoxylin and eosin (H&E) for histopathologic examination.

Statistical analysis

All statistical analyses were perfomed with IBM SPSS statistics for Windows (version 21; SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± SD. Experimental groups were compared using ANOVA (post-hoc Tukey HSD) and Kruskal–Wallis (post hoc Mann-Whitney U) tests. A p value < 0.05 was considered to be statistically significant.

Results

Body (BW) and liver weights, and liver index

BW significantly decreased in experimental hyperthyroidism (T4 group). While liver weight did not changed, the liver index was found to be increased in this group. ALA treatment did not change body and liver weights in T4 treated rats. Only liver index decreased significantly after ALA treatment (Table 1).

Table 1. Efects of ALA on body and liver weights, as well as liver index study groups (mean± SD; n=6 each)

	Control	ALA	T4	T4+ALA
Body weight [g]	310.8±38	319.5±44.3	261.0±17.0[a]	307.3±17.3
Liver weight [g]	7.94±1.21	9.51±1.90	9.58±0.47	8.98±0.66
Liver index* [%]	2.5 ±0.16	2.95±0.22	3.68±0.29[a]	2.92±0.13[b]

α-lipoic acid (ALA), [a] p< 0.05 as compared with control; [b] p< 0.05 compared with T4 group, groups (6 rats of each): Control, ALA, T4 and T4+ALA, *Liver index = Liver weight × 100 / body weight

Serum thyroid function tests

T4 administration significantly increased serum fT3 and fT4 levels. ALA treatment resulted in a significant decrease in fT3 and fT4 levels in hyperthyroid rats (Table 2).

Table 2. Effects of ALA on serum fT3, fT4 levels, ALT and AST activities in study groups (mean± SD; n=6 each)

	Control	ALA	T4	T4+ALA
fT3 [pmol/L]	3.17±0.40	3.56±0.56	11.6±3.51[a]	5.81±1.78[a,b]
fT4 [pmol/L]	26.3±5.90	28.3±5.76	114.8±47.4[a]	50.7±21.5[a,b]
ALT [U/L]	44.6±6.50	55.8±16.7	58.5±10.1	63.0±18.6
AST [U/L]	117.0±16.8	111.8±16.9	119.1±25.8	132.8±28.3

α-lipoic acid (ALA), free T3 (fT3), free T4(fT4), alanine aminotransferase (ALT), aspartate aminotransferase(AST), thyroxine (T4), [a] p<0.05 as compared with control; [b] p<0.05 as compared with T4 group

Serum liver function tests

Serum ALT and AST activities did not change in hyperthyroid rats. No change were observed in ALT and AST activities after ALA treatment in comparisson with hyperthyroid rats (Table 2).

Hepatic prooxidant and antioxidant parameters

There were significant increases in hepatic ROS, MDA, PC levels in hyperthyroid rats (Figure 1). FRAP values significantly increased and GSH levels decreased. However, hepatic SOD, CAT and GSH-Px activities did not alter in hyperthyroid rats (Figure 2). ALA administration significantly decreased ROS, MDA and PC levels [Figure 1]. However, there were no significant differences in hepatic FRAP and GSH levels, SOD, CAT and GSH-Px activities after ALA administration to hyperthyroid rats (Figure 2).

Histopathological evaluation

Normal liver histological structure was seen in control and ALA groups (Figure 3). Necrotic and necrobiotic appearance in liver paranchymal cells, irregularity in lobular structure, vacuolar degeneration in some cells and sinusoidal congestion were observed in T4 group. In T4+ALA group, the liver showed similar appearance like the T4 group, but there was a decrease in parenchymal necrosis and a narrowing in the sinusoids, whereas vacuolar degeneration was not seen. In addition, several apoptotic cells were detected (Figure 3).

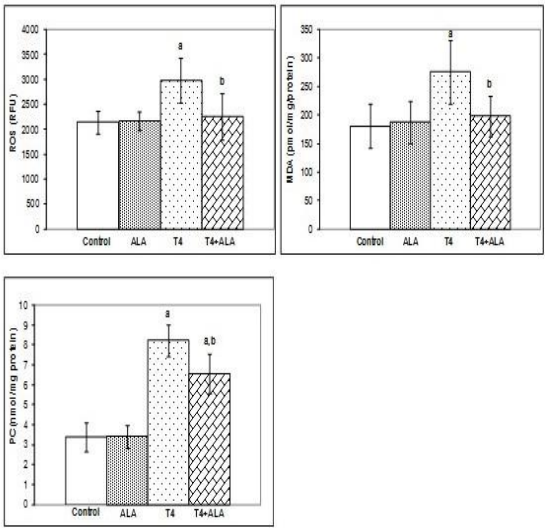

Figure 1. Effects of α-lipoic acid (ALA) on reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl (PC) levels in liver tissue in thyroxine (T4) administered rats (mean±SD; n=6 each).

[a]p<0.05 as compared with control; [b]p<0.05 as compared with T4 group.

Discussion

It is well known that there is a delicate balance between prooxidant and antioxidant systems in the body. The increase of free radicals and/or weakness of antioxidant system results in oxidative stress with subsequent organ damage. Through accelerating the basal metabolism and respiratory chain reactions, thyroid hormones increase generation of ROS in blood and various tissues with subsequent oxidative stress [2-4]. Hovewer, controversial results were obtained from many experimental and clinical studies [2-4, 8, 11, 25, 26]. A lot of factors such as severity of the hyperthyroidism, different susceptibility of various tissues to oxidative stress, and different methods used for the evaluation the prooxidant-antioxidant balance are involved this contradiction [2-4, 8, 11, 25, 26].

Many investigators have investigated the prooxidant-antioxidant balance in body fluids and various tissues of experimental hyperthyroid animals. Because many exogenous compounds are metabolised in liver, due to of its rich mitochondrial content and important role in body metabolism, the liver is among the most frequently studied tissues for the evaluation of prooxidant-antioxidant balance. Several investigators have reported increased levels of hepatic ROS [3, 27], MDA [9, 11, 25], diene conjugate [28], PC [3, 9, 27] and 8-hydroxy-deoxyguanosine [26] levels in rats with hyperthyroidism, however enzymatic and non-enzymatic antioxidants were found to be decreased [6, 25]. In our study, it was found that ROS, MDA and PC levels were also increased,

GSH levels were decreased, and there were no changes in antioxidant enzyme activities in liver. These findings are consistent with the findings obtained in other studies on the same subject [3, 9, 11, 25, 27, 28]. On the other hand, our study

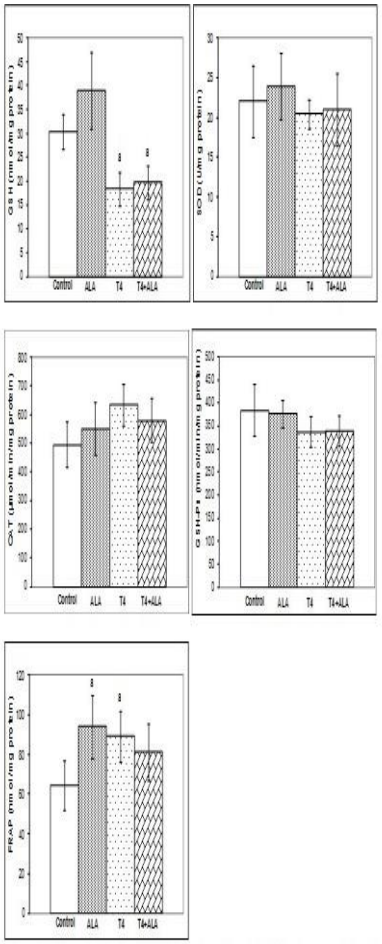

Figure 2. Effects of α-lipoic acid (AL) on glutathione [GSH] levels, superoxidase dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities and ferric reducing antioxidant power (FRAP) values in liver tissue in thyroxine (T) administered rats (mean±SD; n=6 each).
[a]p<0.05 as compared with control; [b]p<0.05 as compared with T4 group.

showed an increase in FRAP levels in the liver. As FRAP is an indicator of total antioxidant power in body fluids and tissues, increased value may be a compensatory mechanism against the oxidative stress developing in the liver in hyperthyroid state.

In recent years, it has been suggested that antioxidant therapy may be helpful in preventing oxidative stress in hyperthyroidism. For this purpose, various antioxidants such as vitamin E, curcumin, and quercetin have been used in hyperthyroidism and some favorable results have been reported [7, 9-11]. ALA is a mitochondrial coenzyme with powerful antioxidant properties. ALA has a beneficial effect in various conditions related with induced oxidative stress. Indeed, many investigators have reported that ALA is effective in ameliorating of various pathologies related with impaired prooxidant-antioxidant balance such as atherosclerosis, metabolic syndrome, diabetes mellitus, and diabetic neuropathy [12-15]. As far as we know, the effectiveness of ALA has not been investigated yet in patients with hyperthyroidism and experimental hyperthyroidism as well. Five weeks durated ALA treatment decreased significantly fT3 and fT4 levels. The suppressing effect of ALA on thyroid hormones levels in the literature was detected for the fist time in our study. The reason of fT4 and fT3 decreasing effect of ALA may be due to the inhibition of thyroid peroxidase and 5'-deiodinase enzymes. As a radical scavenger, by reducing H_2O_2 [which is necessary for function of thyroid peroxidase] ALA may also reduce the activity of thyroid peroxidase in hyperthyroid conditions. ALA administration to the hyperthyroid

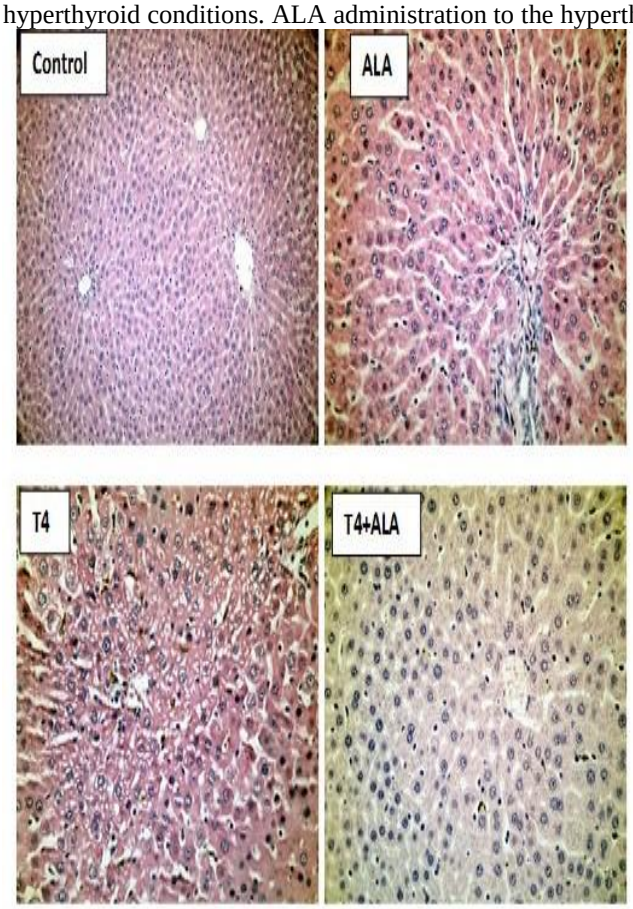

Figure 3. Histopathological findings in the liver tissues of hyperthyroid rats treated with α-lipoic acid (ALA) (H&E × 200).

rats decreased the elevated prooxidant markers in the liver, but not altered the antioxidant system.

Our findings indicate that: a] prooxidant state developed in the liver in experimental hyperthyroidism; b] ALA treatment has improving effect on hyperthyroid state by decreasing the elevated serum fT3, fT4, and by regulatig the prooxidant-antioxidant balance in the liver.

As a conclusion, ALA may be effective supportive agent in the treatment of hyperthyroidism.

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Gastrointestinal findings in children with Down syndrome: Is there an early sign for Celiac disease?

Down sendromlu çocuklarda gastrointestinal bulgular: Çölyak hastalığı için erken bir belirti var mı?

Halil Kocamaz [1], Sedat Işıkay [2]

Abstract

Aim: To investigate the prevalence of celiac disease (CD) among children with Down's syndrome (DS) and its association with gastrointestinal symptoms and other accompanying diseases.
Methods: The study was consisted of regular trisomy 21 patients who were under follow-up in our department. The age, gender, gastrointestinal symptoms (abdominal pain, constipation, diarrhea, abdominal distension, vomiting, flatulence, and unsatisfactory weight gain/weight loss) and accompanying diseases were recorded. Anti-tissue transglutaminase (anti-tTG) immunoglobulin A (Ig A) levels were analyzed in all cases. Serologically positive patients were referred to a pediatric gastroenterologist for intestinal biopsy.
Results: Totally 98 children with a mean age of 3.2±2.81 years (range: 2-13 years) diagnosed with the DS were included in this study. Among study participants, 46 (46.9%) were female. Among study participants, 3 (3.1%) had positive anti-tTG IgA results and endoscopic biopsies revealed the diagnosis of Marsh type 3b CD in all of them. In correlation analysis, hypothyroidism (p=0.03) and presence of diarrhea (p=0.04) significantly correlated with the CD presence among children with the DS. Diarrhea increased the risk for 1.50 times (0.67-3.34) while hypothyroidism increased the risk for 2.75 times (0.55-13.67) among patients with DS.
Conclusion: Clinicians should be aware of an increased prevalence of CD among patients with DS especially in children with diarrhea and/or hypothyroidism.

Key words: Celiac disease, children, Down syndrome

Öz

Amaç: Down sendromlu (DS) çocuklarda çölyak hastalığının (ÇH) sıklığı ile gastrointestinal semptomlar ve eşlik eden diğer hastalıklarla birlikteliğini saptamak.
Yöntemler: Çalışmaya kliniğimizde takip edilen regüler trizomi 21 olan hastalar alındı. Yaş, cinsiyet, gastrointestinal semptomlar (karın ağrısı, kabızlık, diyare, abdominal distansiyon, kusma, şişkinlik ve kilo alımı / kilo kaybı) ve eşlik eden ek hastalıklar kaydedildi. Tüm olgularda anti-doku transglutaminaz (anti-tTG) immünoglobulin A (IgA) düzeyleri analiz edildi. Çölyak serolojisi pozitif saptanan hastalara, çocuk gastroenteroloji uzmanı tarafından ince bağırsak biyopsisi yapıldı.
Bulgular: Çalışmaya yaş ortalaması 3,2 ± 2,81 yıl (2-13 yıl) olan 98 çocuk alındı. Hastaların 46'sı (% 46,9) kızdı. Hastaların 3'ünde (% 3,1) anti-tTG IgA pozitifliği vardı ve endoskopik biyopsilerin hepsinde Marsh tip 3b ÇH tanısı saptandı. ÇH saptanan DS'lu çocuklarda, korelasyon analizinde, hipotiroidizm (p=0,03) ve diyare (p=0,04) görülmesi anlamlı olarak ilişkili idi. DS'lu hastalarda, ishal ÇH görülme riskini 1,5 kat (0,67-3,34) artırırken, hipotiroidizmin 2.75 kat (0.55-13.67) arttırdığı saptandı.
Sonuç: Klinisyenler, DS'li çocuklarda özellikle diyare ve / veya hipotiroidi varlığında ÇH'nı göz önünde bulundurmalıdır.

Anahtar kelimeler: Çölyak hastalığı, çocuk, Down sendromu

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Introduction

Celiac disease (CD) is an immune-mediated systemic disease triggered by gluten in genetically susceptible individuals that is characterized by the presence of specific antibodies. The pathogenesis of CD includes a dominant HLA involvement (DQ2 or DQ8), autoantibodies against transglutaminase and an obviously defined environmental trigger (gluten) [1]. CD has a wide clinical spectrum of gastrointestinal and/or extraintestinal symptoms [2]. Due to its auto immune nature, CD has been associated with other autoimmune diseases including type 1 diabetes mellitus, Hashimoto's thyroiditis and Down syndrome (DS) [3]. Trisomy 21, DS, has been related to high levels of autoantibodies and autoimmune diseases. Autoimmune regulator protein (AIRE) that is located on chromosome 21 has been suggested as the main predisposing factor for autoimmunity in patients with DS [4,5]. There are few study about the association of CD with DS and the prevalence of CD in DS was reported to be between 5-13% in different studies [6-8].

The aim of this study was to investigate the prevalence of CD among children with DS and its association with gastrointestinal symptoms.

Material and methods

Study Design and Patient Selection

This cross-sectional study was conducted between January 2013 and December 2014. The group consisted of regular trisomy 21 patients who were under follow-up in our department. All parents were informed about the investigation. The study was approved by the local Ethics Committee.

Clinical assessment included detailed physical examination, measurement of weight and height plotted on growth carts for DS children followed by an interview of the patients and parents about gastrointestinal symptoms. The age, gender, gastrointestinal symptoms (abdominal pain, constipation, diarrhea, abdominal distension, vomiting, flatulence, and unsatisfactory weight gain/weight loss) and accompanying diseases were recorded.

Venous blood samples (5 mL) were obtained in anticoagulant containing tubes, and anti-tissue transglutaminase (anti-tTG) immunoglobulin A (Ig A) levels were analyzed. The anti-tTG IgA antibodies were investigated through an enzyme-linked immunoassay (ELISA) using human recombinant antigen. Total IgA levels were also studied to determine the IgA deficiency. The patients who has not been introduced gluten are excluded from the study.

Serologically positive patients were referred to a pediatric gastroenterologist for intestinal biopsy. In upper gastrointestinal system endoscopy, three specimens were taken from the duodenal bulb and four from the second part of the duodenum, fixed with 10% formalin and sent to the Pathology Laboratory, where they were processed in paraffin and stained with hematoxylin–eosin. Marsh classification was established for the identification of CD from biopsy specimens [9].

Statistical analysis

Statistical analyses of the data were performed with the SPSS program (Statistical Package for the Social Sciences version 21, Chicago, IL, USA). After testing for normality of the data using Shapiro-Wilk test, one way analysis of variance was used to perform group comparisions. The quantitative variables were described as the mean ± SD and the categorical variables as the frequency and percentage. The study patients with and without CD were analyzed with the descriptive statistics to determine the correlation of accompanying diseases and symptoms with CD and Odd's ratios were calculated for the significantly correlated data. We used Chi-square analysis for to determine relations between variables and Logistic Regression Analysis for to determine Odd's ratio values. A P value <0.05 was considered statistically significant.

Results

Totally 98 children with a median age of 35 months (range= 9 months-156 months) diagnosed with the DS were included in this study. Among study participants, 46 (46.9%) were female. Accompanying symptoms and diseases of the study participants were recorded and 61 (62.2%) of them were having growth retardation, 4 (4.1%) were having recurrent diarrhea, 19 (19.4%) were having constipation. On the other hand, 57 (58.2%) children were having congenital heart disease, 13 (13.3%) of them were having hypothyroidism, 2 (2%) were having biliary stones, 2 (2.0 %) were having undescended testicles, 1 was having ectopic kidney, 3 (3.1%) cases had the history of an operation due to ileus. Two cases were diagnosed with cerebral palsy, and interestingly, epilepsy was determined in 4 (4.7%) of the study participants and 3 of those 4 children were diagnosed with the west syndrome (WS).

Among study participants, 3 (3.1%) had positive anti-tTG IgA results and endoscopic biopsies revealed the diagnosis of Marsh type 3b CD in all of them. None of the patients had IgA deficiency. The general characteristics of these 3 cases are summarized in Table 1.

Table 1: General characteristics of children with Celiac disease

	Case 1	Case 2	Case 3
Age (years)	2	2	3
Gender	Male	Female	Female
Diarrhea	-	-	+
Constipation	-	-	+
Hypothyroidism	+	-	+
Epilepsy	+	-	-
Congenital Heart Disease	+	-	+
Laryngo-malasia	+	-	-
Growth retardation	+	+	+
Iron deficiency anemia	+	+	-

In correlation analysis, hypothyroidism (p=0.03) and presence of diarrhea (p=0.04) significantly correlated with the CD presence among children with the DS, but presence of growth retardation (p=0.25), constipation (p=0.56), congenital heart disease (p=0.59), epilepsy (p=0.14), bile stone disease (p=0.95), ectopic kidney (p=0.95) benign paroxysmal vertigo (p=0.95), Vitamin B12 deficiency (p=0.76), foliate deficiency (p=0.95), and iron deficiency (p=0.74) did not correlate with the CD presence. The odd's ratios for hypothyroidism and diarrhea are summarized in Table 2.

Table 2: Odd's Ratios of Hypothyroidism and Diarrhea

	Odd's ratio	95% Confidence Interval
Hypothyroidism	2.75	0.55-13.67
Diarrhea	1.50	0.67-3.34

Discussion

The prevalence of CD was estimated as 0.47% in healthy Turkish school children [10]. Recently, Kansu et al. [11] reported the overall prevalence of CD as 0.95% in 1047 children with chronic abdominal pain.

The common autoimmune mechanisms in CD and DS propelled the investigators to determine the togetherness of these

2 diseases. Marild et al. [12] reported a 6 fold increased risk of CD in individuals with DS. Nisihara et al. [13] reported the prevalence of CD in patients with DS as 5.6%. Roizen et al. [14] investigated 440 children with DS to determine the frequency of associated medical problems in those patients and reported the prevalence of CD as 5%. Not being too far from these results, we also have determined the prevalence of CD as 3.1% among children with DS. However, Alanay et al. [15] reported that only one patient out of 100 (1%) was detected to be anti endomysial IgA-positive but this child's family refused consent for the biopsy procedure and biopsy could not be performed. Similarly, Pavlovic et al. [16] examined 82 children with DS aged 8 months to 8.6 years for the existence of CD and reported that in 4 children immunoglobulin A and/or immunoglobulin G transglutaminase antibodies were positive, but enteric biopsies showed absence of CD in all cases and the authors suggested not to the screen the children with DS for CD before the age of 8 years. Nevertheless, in our study, all children diagnosed with CD were under the age of 8 years. On the other hand, presence of DS among Celiac patients has also been investigated. In a previous prospective study, we have investigated the neurological findings among celiac patients and reported that 1 (0.3%) out of 297 celiac patients was having DS [17]. Al-Qabandi et al. [18] retrospectively reviewed the records of 47 children diagnosed with CD and reported that 3 (6.4%) of them were also having the diagnosis of DS. Stordal et al. [19] investigated 3006 children with CD for the coexisting conditions and reported that 47 (1.6%) of them were having DS. In general, the prevalence of DS has been reported to be approximately 9-10 Per 10000 live births in Turkey [20] and the prevalence of DS determined in those studies among patients with CD was also rather high.

Interestingly, in another study Bhat et al. [6] reported the prevalence of CD as 7% among 100 children with DS and defined that the pallor and anemia as significant risk factors associated with CD in this group. In this study, for the first time in literature, we have determined that diarrhea or hypothyroidism significantly correlated with the presence of CD among children diagnosed with DS. Diarrhea increased the risk for 1.50 times (0.67-3.34) while hypothyroidism increased the risk for 2.75 times (0.55-13.67) among patients with DS. In fact this finding was not surprising since Hashimoto's thyroiditis is an autoimmune disease that increases the risk of presence of other autoimmune diseases and diarrhea is one of the most common symptoms of CD [21, 22]. Cerqueira et al. [23] reported the prevalence rate of CD as 9.2% in adults with DS.

Remarkably in this study, we have determined that epilepsy was accompanying DS in 4 (4.7%) children and 3 of those 4 children were diagnosed with the WS. In literature, Barca et al. [24] reported that 9 (23%) of 39 children with DS were also having epilepsy and similar with our results in that study the most frequent epileptic syndrome associated with DS was reported as WS. Lack of a control group and low number of patients are the main limitations of this study.

In this study, we have determined an increased prevalence of CD among patients with DS compared with the general population. Moreover, presence of diarrhea and/or hypothyroidism was associated with the CD. In that aspect, clinicians should be aware of an increased prevalence of CD among patients with DS especially in children with diarrhea and/or hypothyroidism.

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A case of cutaneous pseudolymphoma induced by medicinal leech application and review of the literature

Tıbbi sülük uygulaması sonucu ortaya çıkan kutanöz psödolenfoma olgusu ve literatürün gözden geçirilmesi

Habibullah Aktaş [1], Aziz Ahmad Hamidi [2], Gökşen Ertuğrul [1], Harun Erol [3]

Abstract

Medicinal leech application is a common traditional therapy used for various disorders in many cultures for thousands of years. Although it is usually a safe method, serious side effects can also be seen. As a rare side effect of this method, cutaneous pseudolymphoma is a reactive skin disease caused by foreign antigens, infectious processes and arthropod bites. Idiopathic cases sometimes can be seen.

In this article, we present a case of cutaneous pseudolymphoma in an elderly woman with multiple nodules over her lumbar region emerging after leech application.

Keywords: Medicinal leeches, pseudolymphoma, traditional methods.

Öz

Tıbbi sülük uygulaması binlerce yıldır birçok kültürde çeşitli hastalıkların tedavisinde kullanılan yaygın bir geleneksel tedavi yöntemidir. Genellikle güvenli bir yöntem olmasına rağmen, bazen ciddi yan etkiler de görülebilir. Bu metodun nadir bir yan etkisi olarak kutanöz psödolenfoma, yabancı antijenler, enfeksiyöz süreçler, artropod ısırıkları ve bazen idiopatik nedenlerle oluşan reaktif bir deri hastalığıdır.

Bu yazıda, sülük uygulaması sonrası çok sayıda nodülü olan ve kutanöz psödolenfoma tanısı konulan bir kadın olguyu sunuyor ve literatürü gözden geçiriyoruz.

Anahtar kelimeler: Psödolenfoma, tıbbi sülük, geleneksel yöntemler.

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Informed Consent: The written consent was received from the patient who was presented in this study.
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Introduction

Medicinal leech application (MLA) is a known traditional therapy in the eastern countries for thousands of years for various disorders [1].The traditional therapies have been accepted as legal by Ministry of Health in Turkey for a couple of years. This legalization led to a significant increase in the number of applications of traditional methods including MLA in this country.

Lymphocytoma cutis or pseudolymphoma is a reactive skin disease, sometimes named as cutaneous lymphoid hyperplasia. The disease manifests itself as single or multiple red or purple papules, nodules or plaques mostly over the face, but the lesions are also involved in other body regions. There is a dense lymphocyte accumulation within the dermis histologically [2].The causative factors include foreign antigens, infectious processes and arthropod bites. Idiopathic cases sometimes can also be seen. A detailed history is very helpful in differential diagnosis of cutaneous pseudolymphoma. Adnexal tumors, granuloma faciale, skin tuberculosis, rosacea, sarcoidosis and other granulomatous disorders should be thought for differential diagnosis of the disease.

We present a case of cutaneous pseudolymphomas caused by MLA.

Case report

A 65-year-old woman was admitted to our clinic due to rash with severe itchiness in the lumbar region. On skin examination, we observed eight semi-hard purple nodules over her lumbar area (Figure 1). There was no pain on palpation. In detailed history, we learned that she put eight leeches over her lumbar area for a lumbar pain. Excisional biopsy was performed for one of the lesions. Histopathological examination yielded dense polyclonal lymphocyte infiltration in the superficial and deep dermis, compatible with the diagnosis of benign cutaneous lymphoid hyperplasia (Figure 2). In hematological and radiological investigation, we did not see any abnormalities suggestive of lymphoma. We administered 20 mg/ml intralesional triamcinolone with 30 seconds open spray freeze (cryotherapy) per lesion with liquid nitrogen. There were residual hyperpigmented macules over the disordered area with no subjective symptoms after three weeks.

Written consent was taken from the patient.

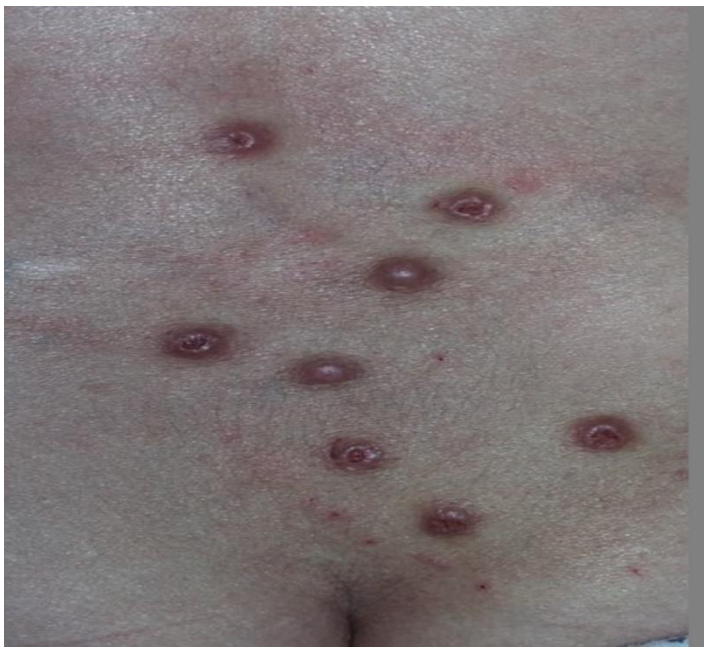

Figure 1: Purple nodules over the lumbar region.

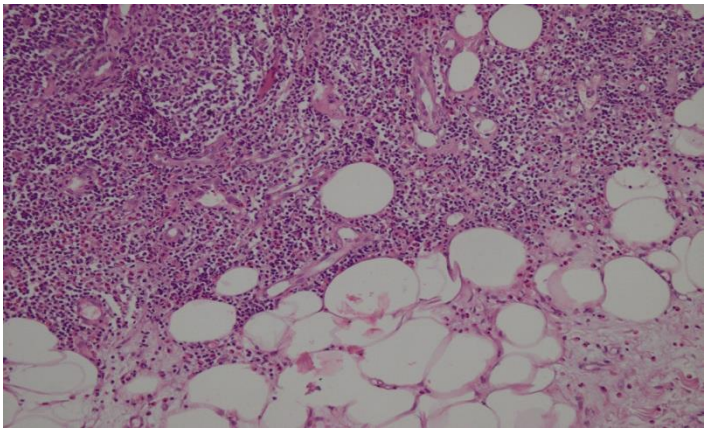

Figure 2: Pathological image shows dense lymphocytic infiltration in the dermis.

Discussion

MLA is an auxiliary method which is mainly used in association with vascularization. MLA has been found to be an effective medication in salvage of venous congestion of free flap by increasing its revascularization [1]. However, it also carries the significant risk of contamination of severe infectious agents to the host. Life-threatening infectious diseases such as sepsis and meningitis have also been reported [3-5]. Prolonged bleeding is another dangerous complication of medicinal leech therapy [6]. Cutaneous pseudolymphomas caused by MLA have been rarely reported in the literature. The summary of reported cases are shown in Table [2, 7-10]. Among the cases, there was only one male patient, the others were women. Probably the women are more interested in such traditional treatments. In female patients, the leech application was performed due to pain, contrary to an esthetic reason for the male patient [2, 7-10].

Histopathology is the gold standard in the diagnosis of cutaneous pseudolymphoma together with a detailed patient history. If detailed history is not taken, etiology could be missed because the lesions usually occur at least one month after MLA.

This case report may be regarded as a public health alert about uncontrolled traditional treatment methods. Increase in MLA may lead to life-threatening events such as severe infections and uncontrolled bleedings apart from cutaneous lymphoid hyperplasia which is a relatively benign side effect compared with others. We recommend that the authorities inform public about traditional treatment methods, including medical leeches, and that they carefully monitor inappropriate practices which are not made by physicians.

Table. Pseudolymphoma cases reported in the literature.

Article	Age (year)	Gender	Localization	*Duration after MLA	Reason for MLA	Treatment
Smolle J 2000	56	F	Lower legs	After several weeks	Venous insuff.	IL steroid
Choi Y 2012	52	M	Lower eyelids	After several months	Infra orbital dark circles	IL steroid
Khelifa E 2013	77	F	Lower back	After several months	Lumbar pain	Topical and IL steroid
Altamura D 2014	50	F	Back	After 5-6 weeks	Fibro myalgia	Topical steroids
Topiko-wska M 2018	38	F	Pubic region	Unclear	Uterine myoma	Topical and IL steroid
Our case	65	F	Lower back	After a few months	Lumbar pain	IL steroid

*Time to development of lesions after leech application, MLA: Medicinal leech application, IL: intralesional.

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Choanal polyp originating from the middle turbinate: A case report

Orta konkadan kaynaklanan koanal polip: Bir olgu sunumu

Süha Ertuğrul [1]

Abstract

Choanal polyps are most frequently sinusal in origin and a great majority of them are in the form of antrachoanal polyps. Apart from that, atypical localization sites including the superior turbinate, septum, cribriform plate have rarely been reported. Cases of choanal polyps originating in the middle turbinate are very rare. This paper reports a concha-choanal polyp case originating from the middle turninate, which was excised via endoscopic surgical technique, in the light of clinical and radiological findings.

Key Words: Endoscopic sinus surgery, polyp, choana, concha, middle turbinate.

Öz

Koanal polipler sıklıkla sinüs orjinlidir ve bunların da büyük kısmı antrakoanal polip şeklindedir. Bunun dışında süperior konka, septum, kribriform plak gibi atipik lokalizasyonlar nadiren raporlanmıştır. Orta konka kaynaklı koanal polipler çok nadirdir. Bu yazıda endoskopik cerrahi teknik ile eksize edilen orta konkadan köken alan konka-koanal polip olgusu klinik ve radyolojik bulgular eşliğinde sunuldu.

Anahtar Kelimeler: Endoskopik sinüs cerrahisi, polip, koana, konka, orta turbinat.

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Conflict of Interest: No conflict of interest was declared by the authors.
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This case has been presented as a poster presentation in 12th International ORL-HNS Congress.
Bu olgu 12. Uluslararası ORL-HNS Kongresi'nde poster olarak sunulmuştur.

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Introduction

Choanal polyp (CP) can be defined as benign, solitary, inflammatory soft tissue masses that extend towards the nasal cavity and the nasopharynx. CPs usually originate from the maxillary sinus, unusual origins, such as the middle turbinate, the ethmoid sinus, the nasal septum, the inferior concha, the sphenoid sinus, hard and soft palate, have been reported in the literature [1-3]. A CP originating from the middle turbinate is an extremely rare entity.

Herein, we present a rare case of CP, which originated from the posterior end of the middle turbinate in the light of clinical and radiological findings.

Case report

A 78-year-old male patient presented to our clinic with the symptom of nasal obstruction that had been present for 30 years. He had no history of allergy. Anterior rhinoscopy revealed a single polypoid mass on the right side. Nasal endoscopic examination revealed a CP, originating from the posterior side of the right middle turbinate (Figure 1). In his computerized tomography (CT) and magnetic resonans images, it was observed that his soft tissue density started at the level posterior end of middle turbinate and extended to the choana (Figure 2, 3). His paranasal sinus aerations were natural. Considering the patient's age and absence of complete obstruction of the choana by the polyp, non-surgical follow up was recommended at first. However, due to the decision of the patient, surgical treatment was planned.

Endoscopic endonasal sinus surgery was performed under local anaesthesia. At the operation, removal of the CP and partial resection of the middle turbinate were performed. No post-operative complications were observed. No recurrence was observed in the 1-year-long follow-up of the patient.

Written informed consent was obtained from the patient.

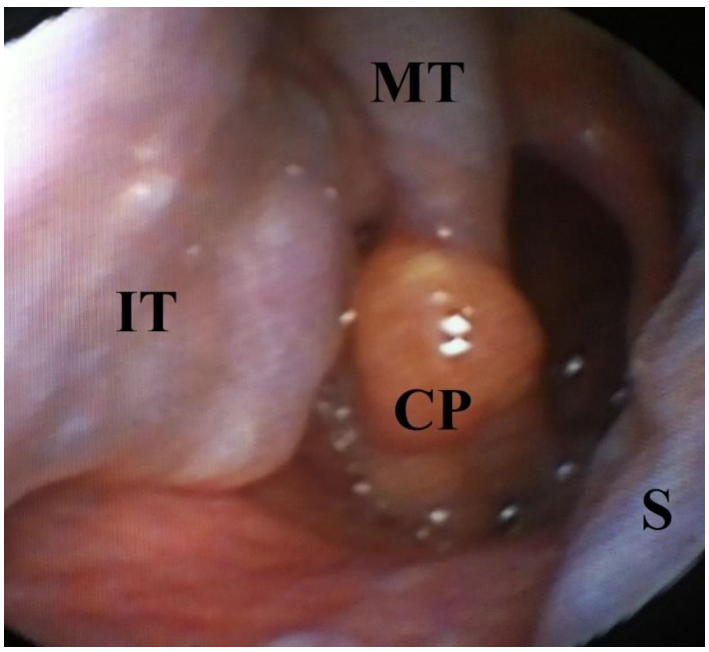

Figure 1. Endoscopic image of the right nasal cavity (CP: choanal polyp, IT: inferior turbinate, MT: middle turbinate, S: septum).

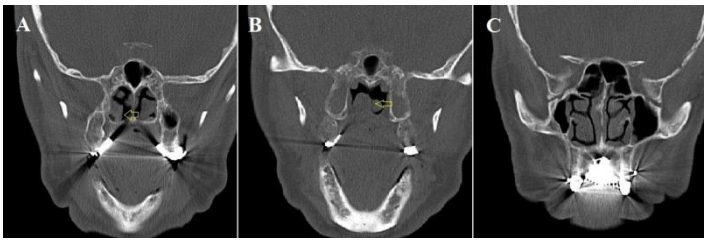

Figure 2. Computed tomography image A. Paranasal tomography shows the site of attachment of the polyp to the middle turbinate in the coronal section B. Choanal portion of the polyp. C. Aeration of the paranasal sinuses was normal.

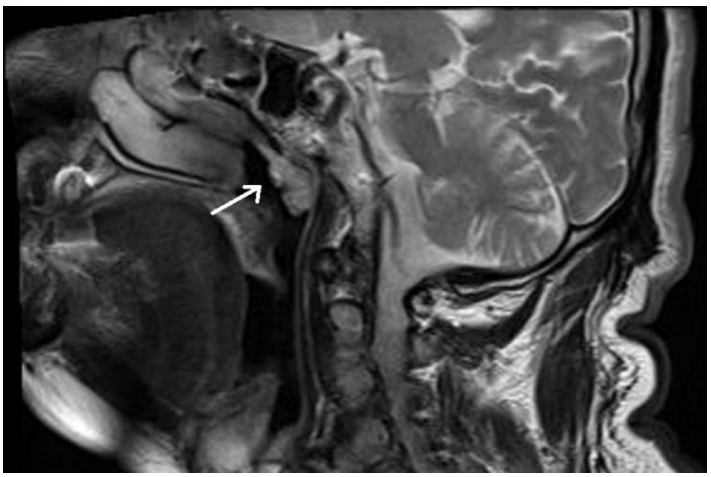

Figure 3. Magnetic resonance image shows the soft tissue density (arrow) started at the level posterior end of middle turbinate and extended to the choana.

Discussion

CP was originally described by Killian in the year 1906 [4]. However, its etiology has not yet been fully established. Local reasons such as ostium obstruction and chronic sinusitis are considered at the forefront in the etiology. Chronic inflammation and negative pressure occurring in the antrum following ostium obstruction result in the development of a mural cyst at this site. Berg et al. identified that the expansion of an intramural cyst into the nasal cavity was important in the development of CP [5]. According to Myers, antrochoanal polyps (ACP) in children develop in association with bacterial infection and cystic fibrosis [6]. The association between allergy and ACP is not clear. Our patient did not have history of allergy; therefore, a skin prick test was not conducted.

Nasal obstruction is the most frequently observed symptom in CP patients. Furthermore, they may also develop snoring, sleep apnea, headache, feeling of a foreign body in the throat and swallowing problems [7]. Our case only had nasal obstruction.

CPs originate most frequently in the maxillary sinus whereas they may also originate in the sphenoid, ethmoid or frontal sinus. However, CPs of extrasinusal origin are rather rare. In 1906, Killian described the first case of CP, which originated from the posterior end of the middle turbinate [4]. Lopatin et al. [8] on the other hand, reported another CP originating from the lateral aspect of the head of the middle turbinate, which upon histopathological examination turned out to be an inverted papilloma.

Nasal endoscopy and CT are mostly adequate in diagnosis. In our case, it could be seen under nasal endoscopy

that the CP originated from the posterior end of the middle turbinate. As for the CT, it is typical with CP to see a one-sided soft tissue density in the nasal cavity and choana. However, in cases where soft tissue density is observed in the nasal cavity and choana whereas the aeration of paranasal sinuses is normal, an atypically localized CP should be suspected as in our case. In differential diagnosis, mucoceles, inverted papilloma, juvenile angiofibroma, olfactory neuroblastoma, nasopharyngeal malignancies, adenoid hypertrophy, nasal polyposis, meningoencephalocele and turbinate hypertrophies should be considered [9].

Endoscopic sinus surgery is the effective method in both diagnosis and treatment. Excision of the diseased mucosa along with the polyp is of great importance for the prevention of recurrences [8]. Our patient had an age of 78 and the polyp could not completely close the choana, so our patient could be followed without surgery. But the patient decided to undergo operation under local anesthesia because he wanted to get rid of nasal obstruction. In our patient, a partial resection of the middle turbinate was performed in order to clean the diseased mucosa along with the CP. No recurrence was observed in his postoperative 1-year follow-up.

In conclusion, a CP of atypical localization should be suspected in one-sided polyps especially if the paranasal sinus aerations seen in CT are normal and it should be taken into consideration that a polyp may also originate in the middle turbibnate.

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DOI: 10.25000/acem.450495 Olgu Sunumu / Case Report

Left atrial extension of metastatic renal cell carcinoma via pulmonary vein: A case report

Metastatik renal hücreli karsinomun pulmoner ven yoluyla sol atriyuma uzanımı: Bir olgu sunumu

Mehmet Şeker [1], Cengiz Erol [1]

Abstract

Cardiac involvement from renal cell carcinoma is rare but well-recognized entity with several cases reported in literature. In majority of cases, the tumor reaches the right heart cavities through a neoplastic thrombus within inferior vena cava. Other mechanisms are dissemination via hematogeous spread and direct extension from either mediastinal or lung lesions involving pulmonary veins. Left heart involvement from renal cell carcinoma with normal inferior vena cava is extremely rare. Direct extension of metastatic pulmonary mass to left atrium from renal cell carcinoma via pulmonary veins is even rarer.
We report a case of left atrial extension via pulmonary vein from metastatic renal cell carcinoma who presented with left cerebellar and right thalamic acute ischemic lesions along with multiple metastatic lesions.

Keywords: Renal cell carcinoma, pulmonary vein, tumor embolus, left atrium.

Öz

Renal hücreli karsinomun kardiyak tutulumu nadir ancak iyi bilinen bir olgudur ve literatürde bir çok vaka bildirilmiştir. Tümör çoğu durumda, sağ kalp boşluklarına vena kava inferiyor içinde neoplastik trombüs yoluyla ulaşır. Bu mekanizma dışında, kardiyak tutulum, hematojen yolla olabileceği gibi, mediyastinal veya akciğer lezyonlarının pulmoner venler yoluyla direk yayılımı ile de ortaya çıkabilir. Ancak vena kava inferiyor tutulumu olmaksızın tümörün sol kalbe ulaşması son derece nadirdir. Renal hücreli karsinomun akciğer metastazlarının, pulmoner venler yoluyla sol atriyuma direk uzanımı daha da nadirdir.
Bu makalede, çok sayıda metastatik lezyonu olan ve sol serebellar ve sağ talamik akut iskemik lezyonlara bağlı belirtileri ortaya çıkan, pulmoner ven yoluyla sol atriyuma direk uzanımı olan metastatik renal hücreli karsinom olgusunu sunuyoruz.

Anahtar Kelimeler: Renal hücreli karsinom, pulmoner ven, tümör embolisi, sol atrium.

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Introduction

Left atrial extension of primary pulmonary tumors via pulmonary veins have been well documented and a number of such instances have been reported in the literature [1, 2]. But, metastatic pulmonary tumors extending to the left atrium via the pulmonary veins are extremely rare [3, 4].

To our knowledge, the case we present here is the fourth citing left atrial extension via pulmonary vein from metastatic renal cell carcinoma (RCC) [5, 6]. In this report, we will briefly review the epidemiologic and clinical features, imaging modalities and current therapeutic strategies.

Case report

A 37-year-old patient was admitted to our hospital for follow-up for RCC. He was diagnosed with RCC and had underwent left radical nephrectomy for limited stage RCC six years ago. After his first operation, he had undergone metastasectomy for right adrenal gland metastasis and then lobectomy for right lung metastasis. He was free of disease for five years.

For evaluation of the current status of the disease, positron emission tomography (PET-CT) revealed a large left lower lung mass and muscular metastasis and cranial magnetic resonance imaging (MRI) revealed multiple infratentorial and supratentorial metastatic lesions. Radiotherapy was added to his therapy for cranial metastasis.

After 4 months, he presented with numbness and left cerebellar and right thalamic acute ischemic lesions along with multiple metastatic lesions.

Echocardiography showed a mass in the left atrium that extends to the left inferior pulmonary vein (Figure 1). Although, the appearance was consisted with atrial thrombus, the presence of widespread RCC raised the suspicion of metastatic mass. In order to make differential diagnosis cardiac MRI and for probable cardiovascular surgery coronary computed tomography (CT) was performed. Coronary CT (Figure 2) and cardiac MRI (Figure 3) revealed a cavitary left lower lung mass showing contrast enhancement extending to the left atrium via the left inferior pulmonary vein. Cine MR images demonstrated that the mass protrudes towards the atrial outflow (Figure 4). Inferior vena cava (IVC) and the right heart chambers were normal.

Owing to multiple unresectable metastasis and high mortality and morbidity risks, cardiovascular surgery did not offer any intervention and the clinicians decided to continue therapy with a tyrosine kinase inhibitor (axitinib, Inlyta®, Pfizer Pharmaceuticals) and to add anticoagulant agent.

Written consent was taken from the patient.

Discussion

Although cardiac involvement from RCC is rare, it is well-recognized entity with several cases reported in recent literature [4, 7, 8]. In majority of cases, the tumor reaches the right heart cavities through a neoplastic thrombus within IVC. RCC extends into the renal vein and IVC in about 5% to 15% patients and into the right atrium in about 1% of patients [7, 9, 10]. Other mechanisms are dissemination via hematogeous spread and direct extension from either mediastinal or lung lesions involving pulmonary veins. But, cardiac involvement without IVC involvement is extremely rare [8]. In the literature, 31 cases with left heart involvement from RCC with normal IVC was reported [4]. Only 3 of these cases have left atrial involvement from RCC and these 3 metastasis spread hematogeneously. Extension of metastatic pulmonary mass from RCC via pulmonary veins is even rarer [5].

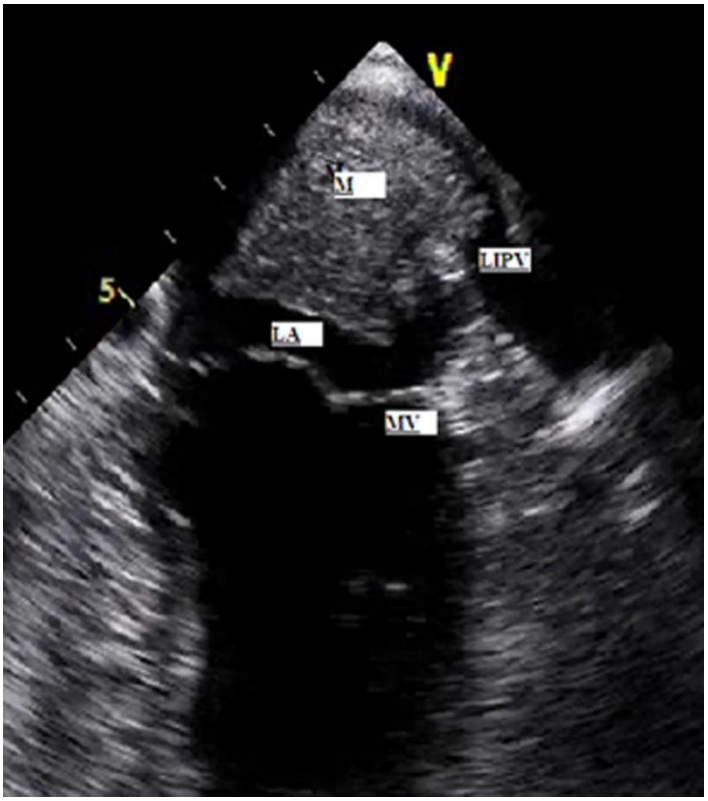

Figure 1. Transesophageal echocardiogram shows a mass (M) in the left atrium (LA) extending to the left inferior pulmonary vein (LIPV). Mitral valve (MV) is normal.

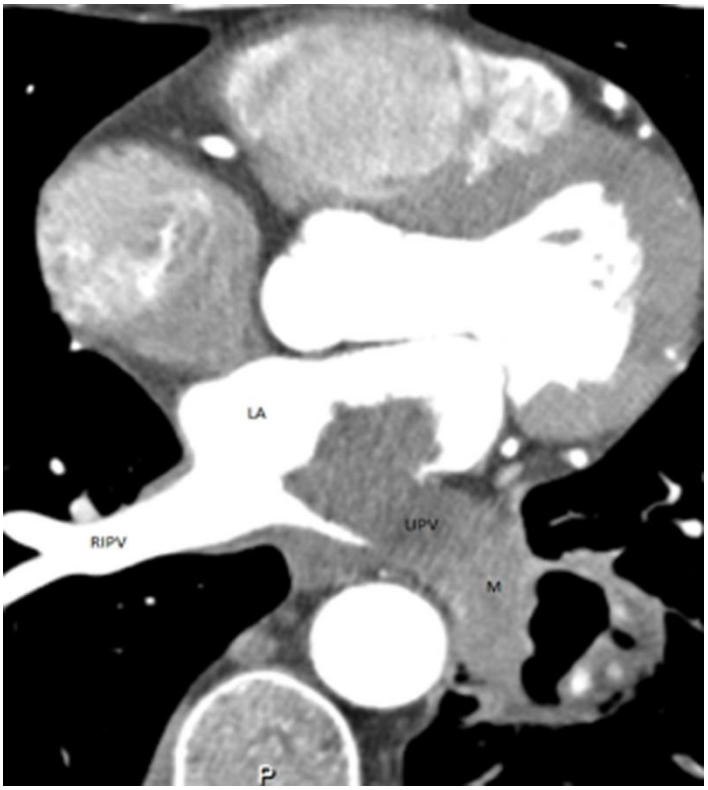

Figure 2. Axial contrast-enhanced CT shows a cavitary mass (M) involving the left lower lobe and extending into the left atrium (LA) through the left inferior pulmonary vein (LIPV). Right inferior pulmonary vein (RIPV) is normal.

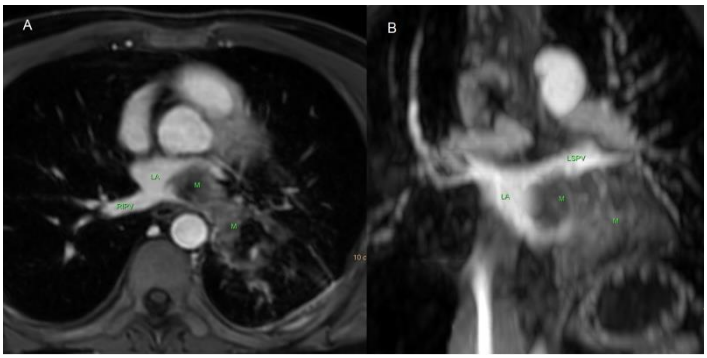

Figure 3. Axial (3a) and coronal-oblique (3b) contrast-enhanced fat saturated T1A demonstrates a contrast enhancing mass (M) in the left lower lobe extending to the left atrium (LA) via the left inferior pulmonary vein and normal right inferior pulmonary vein (RIPV) and left superior pulmonary vein (LSPV).

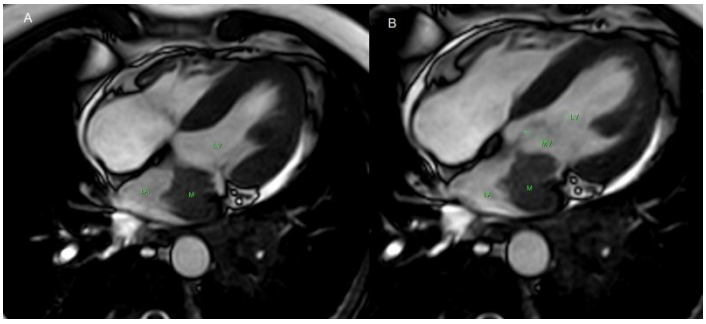

Figure 4. Axial BTFE (balanced turbo field echo) images during systole (4a) and diastole (4b) reveals a mass (M) in the left atrium (LA) protruding towards the mitral valve (MV). LV: Left ventricle.

Most cardiac metastases are clinically silent and may also present with dramatic manifestations that depend on their location and tumor size. Intra-cavitary metastases can cause right and left ventricular or atrial outflow tract obstruction and patients may represent with cardio embolic complications including stroke from left-sided cardiac metastasis or pulmonary emboli from right-sided cardiac metastasis.

Traditionally echocardiography is used as the first-line diagnostic test when there is a doubt for a cardiac mass. But, tissue characterization by means of echocardiography is limited [11].

The technologic advances in CT and MRI have resulted in improvements in imaging of cardiac structures and now, they are widely used as a diagnostic modality to assess cardiac masses. These modalities offer information about morphologic characteristics (location, size, infiltrative nature, presence of pleural/pericardial effusions) and vascularity of the mass with contrast enhancement which can be used to predict the malignancy probability of a cardiac mass [11, 12, 13].

In recognition of its diagnostic capabilities, cardiac MRI is now widely used as a primary imaging technique in the work-up of cardiac tumors [11]. CT offers an alternative second-line imaging strategy where cardiac MRI is not available or is contraindicated and when echocardiography alone has not been sufficient to fully assess a mass.

Most patients who develop cardiac metastasis already have disseminated extra cardiac involvement by the time of diagnosis and recognition of the process is usually not a problem. But, diagnosis may be problematic when the cardiac involvement is isolated. The primer differential diagnosis is thrombus. In such situations, cardiac MRI is indicated to differentiate between a cardiac tumor and an intra-cardiac thrombus. At MRI, the signal intensity of a thrombus can vary with the age of the thrombus. A fresh thrombus will have increased signal intensity on both T1- and T2-weighted images and older thrombus will be hypointense on both T1- and T2-weighted images. Infusion of gadolinium-based contrast material will also allow differentiation between tumors and intracavitary thrombus. Although, making this distinction may prove more difficult in the presence of an old thrombus [12].

Metastasis is a strong predictor in patients with RCC like other malignancies and cardiac metastasis poses therapeutic challenge. They are most often found in patients with multiple metastases and therefore, the most important goal of intervention is palliation of symptoms and improve quality of life, and prolong survival.

In fact, for many patients, surgical resection is not a viable option as a result of tumor location or medical co-morbidities. Surgical resection is generally reserved for isolated cardiac metastasis, for patients in whom complete resection is technically feasible and for cases in which prognosis is otherwise good.

Similarly, treating metastatic RCC with cytotoxic agents and hormones have no rewarding results. But, as the biology of RCC became gradually understood, new therapeutic agents (tyrosine kinase inhibitors, the monoclonal antibody, and mammalian target of rapamycin inhibitors) have been developed and offer much improved progression-free survival [8].

In conclusion, the possibility of cardiac metastasis should be considered in any patient with a malignancy who develops new cardiac symptoms. Cardiac CT and MRI can be used as diagnostic tools with confidence.

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Surgical treatment of bladder diverticulum with radical prostatectomy. A case report and literature review

Radikal prostatektomi ile birlikte mesane divertikülü cerrahisi: Bir olgu sunumu ve literatür derlemesi

M. Yılmaz Salman [1], Adem Fazlıoğlu [2], Fatih O. Kurtuluş [3], S. Erdinç Ünlüer [4]

Abstract

Bladder diverticulum is an important health problem and can cause urinary system dysfunctions and recurrent urinary infections in patients as complications of incomplete bladder emptying. Diverticulas are often accompanied by bladder outlet obstructions and are less likely to be seen with the prostate cancer. This rare condition necessitates advanced surgical planning.
In this report, we wanted to show that radical prostatectomy can be successfully performed with diverticulectomy.

Keywords: Bladder diverticulum, prostate cancer, radical prostatectomy, surgery

Öz

Mesane divertikülleri önemli bir sorun olup, idrarın tam boşalmaması nedeniyle üriner sistem işlev bozukluklarına ve tekrar eden üriner enfeksiyonlara neden olabilir. Divertiküllere çoğu zaman mesane çıkım obstruksiyonları eşlik etmekte olup, prostat kanseri ile birlikte görülme ihtimali daha düşüktür. Nadir olan bu durum önemli bir cerrahi planlama gerektirir.
Sunduğumuz bu vakada, divertikülektomi ile birlikte radikal prostatektominin başarılı bir şekilde uygulanabileceğini göstermek istedik.

Anahtar Kelimeler: Mesane divertikülü, prostat kanseri, radikal prostatektomi, cerrahi

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Introduction

Urinary bladder diverticulum (UBD) may be congenital or acquired. Genetic disorders such as Ehlers Danlos or Williams Beuren syndrome have been associated with UBD [1]. They are mostly acquired and secondary to bladder outlet obstructions in adults. UBD occur in adults with a male to female ratio of 9:1 [2]. It is called as Hutch diverticula if it is located in peri ureteral orifice. The first bladder diverticulectomy was performed by Czerny in 1897 [3].

Treatment of acquired UBD consists of diverticulectomy and relief of the bladder outlet obstruction with open, endoscopic, laparoscopic and robotic approach.

Adult bladder diverticulum with the presence of prostatic adenocarcinoma represents a rare clinical finding. In this paper, we aimed to report a rare association of the bladder diverticulum with prostatic adenocarcinoma in an adult patient.

Case report

A 70-year-old male admitted to our clinic with the complaint of incomplete emptying and decreased urinary flow. The patient had lower urinary tract symptoms for the last three years but his symptoms increased for the last several days. Only hypertension was present in his medical history. No previous surgery was done. International prostate symptoms score (IPSS) score was 25 and international index of erectile function (IIEF) score 18. Prostate specific antigen (PSA) was 5.4 ng/ml. A hard prostate nodule was felt in the left lobe during digital rectal examination. Transrectal ultrasound guided tru-cut prostatic biopsy was performed. Prostatic adenocarcinoma Gleason 7 (4+3), 9 out of 12 core was detected. Computed tomography (CT) scan of the abdomen and the pelvis revealed bilateral grade two hydroureteronephrosis and the well-distended bladder, with a significantly thickened and irregular wall with the presence of two diverticula with the presence of a 12 cm diverticulum at its left wall and 9 cm on base and also detected almost 600 cc residual urine (Figure 1, 2). Maximum flow rate (Qmax) in uroflowmetry was 6.4 ml/s. Prostatic enlargement of the median and the lateral lobes was detected in diagnostic cystoscopy. The bladder had trabeculations and a big diverticulum on the posterior wall. The other diverticulum on the right side of the bladder wall is smaller. There was no abnormal appearance in both diverticula. Urodynamic evaluation was not performed.

Open radical prostatectomy with diverticulectomy was performed due to pre-operative obstructive findings. Via the lower infra--umbilical median incision, the bladder was dissected from its neighborhood tissues and opened. Bilateral ureteral catheters inserted and both diverticula was removed with intra and extravesical approach. Openings of the diverticula was closed with double layer technique. Afterward, external oblique fascia was opened, puboprostatic ligaments were cut. The venous plexus was sutured and cut. Urethra was cut at the apex level and the bladder neck was opened. Seminal vesiculates were dissected. Urethrovesical anastomosis was done. The operation time was approximately 215 minutes. Total blood loss was 150 cc. The patient was discharged on 5th day with urethral catheter. The catheter was removed at the postoperative 18th day.

Pathology report revealed adenocarcinoma of Gleason 7 (4+3) and negative surgical margin. Postoperative 1st month follow up revealed no hydronephrosis with minimal residual urine seen on ultrasonography. Q-max on uroflowmetry increased from 6.4 to 11ml/s. Postoperative PSA was 0.039 ng/ml.

Postoperative 3rd month follow up revealed continence with the use of duloxetine (Nexetin ®, Nobel Pharmacy, Turkey) 40 mg twice a day IPSS score was 4 and IIEF score 8. Tadalafil 5mg (Cialis ®, Lilly, Turkey) was given daily once.

Written consent was taken from the patient.

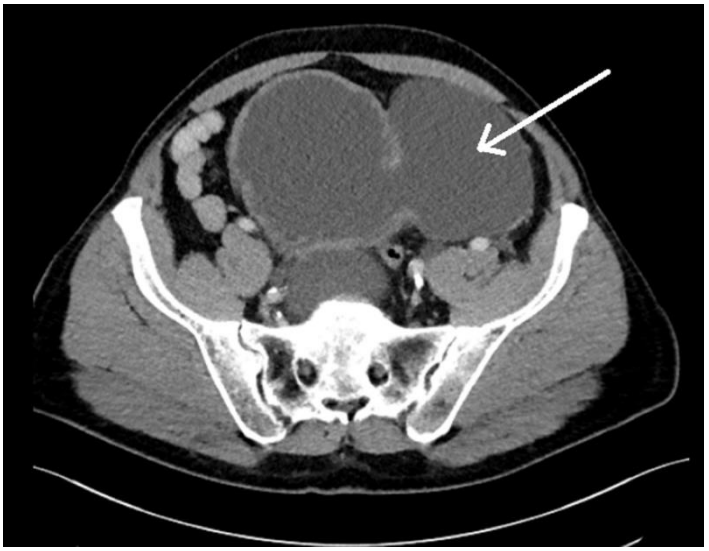

Figure 1. An axial computed tomography scan showing the diverticulum located at the left wall of the urinary bladder (white arrow).

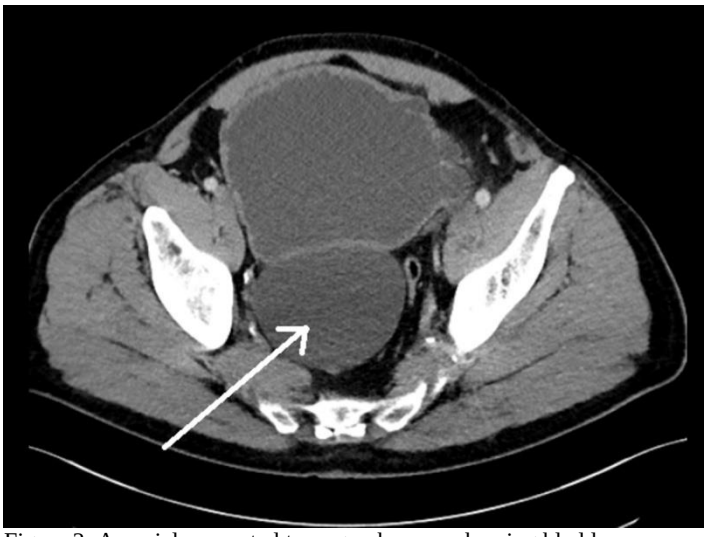

Figure 2. An axial computed tomography scan showing bladder diverticulum located at the base of the urinary bladder (white arrow).

Discussion

Bladder diverticula generally can be described as herniation of bladder mucosa through the weak parts of the detrusor muscle. It was reported first in 1700 by Bartholin, who found the bladder diverticulum in a large scrotal hernia during an autopsy session. In 1730, Morgagni suggested that the bladder diverticulum could result from bladder neck obstruction due to prostate enlargement [4]. Bladder diverticula can cause irritative voiding symptoms and recurrent urinary infections in patients as results of incomplete bladder emptying. Among the treatment modalities, open, endoscopic, laparoscopic and robot assisted surgical techniques can be used. In this report we mention also that these techniques are applicable in the presence of small number of additional surgical pathologies referring to literature. Laparoscopic diverticulectomy was first reported by Parra et al. [5] in 1992 and the first robot assisted technique by Myer et al. [6] in 2007. Since pneumoperitoneum flattens diverticula in these approaches which makes diverticula impossible to recognize, various techniques have been applied to dissect it safely. Moore et al. [7] used methylene blue to identify abdominally the diverticular neck. Myer and Wagner [6] inserted angiographic occlusion balloon catheter into the diverticulum

and inflated the balloon. Parra et al. [5] inserted a flexible cystoscope into the diverticulum and performed transillumination with light source.

In the literature, few examples of combined surgery for bladder outlet obstruction were reported. The first cases of bladder diverticula operation with retropubic prostatectomy were reported by Couvelaire in 1948 and Findlay in 1954 [4]. Propglia et al. [8] and Abdel-Hakim et al. [9] does not recommend the use of transurethral resection of prostate (TURP) and diverticulectomy at the same session. These authors claimed that postoperative continuous bladder irrigation would damage bladder sutures. In 2008, Magera et al. [10] performed simple prostatectomy with robot assisted diverticulectomy having postoperative two days hospital stay and fourteen days catheterization time. Tufek et al. [11] in 2016, studied 9 patients undergoing surgery, combination of TURP and photo selective vaporization of prostate for bladder outlet obstruction with robot assisted diverticulectomy. According to the authors, postoperative continuous bladder irrigation was not needed after operation due to combination of TURP and PVP. Their complication rate was generally lower than patients who received two different surgeries at two separate times and that it was more favorable in terms of cost and duration of hospital stay.

Skolarikos et al. [12] reported 11 cases of radical prostatectomy with diverticulectomy in 2007 as a safe and effective procedure. According to their results, all patients was continent and had improved Qmax value. Similarly Loran et al.[13] and Plomidis et al. [14] reported radical prostatectomy with diverticulectomy as a safe procedure with good oncologic and functional results. We planned radical prostatectomy with diverticulectomy after informing the patient. Operation time was 215 minutes and blood loss was 150 ml approximately. Myer et al. [6] reported operation time as 178 min in five patients in robot assisted diverticulectomy series. Similarly, Altunrende et al. [15] reported operation time as 232 minutes, average blood loss as 100 ml and hospital stay as 3 days in robot assisted diverticulectomy series. In terms of operation time, blood loss and hospital stay, radical prostatectomy with diverticulectomy operation can be applied.

More detailed information may be acquired with prospective series study, not case-report; this is may be lack of paper. Deficiency of urodynamic evaluation may be lack of this case. In this case, we wanted to show that radical prostatectomy can be successfully performed with diverticulectomy. Applying the same seen to appropriate patients will result in satisfactory results both in terms of cost and length of hospital stay, as both are a major surgical procedure and when applied separately will require longer hospitalization and longer recovery times.

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A rare presentation of anthrax with sepsis: A case report

Şarbon'un sepsis ile seyrettiği nadir görülen bir durum: Bir olgu sunumu

Mahmut Dülger [1], Kenan Murat [2]

Abstract

Anthrax is a zoonotic infection caused by Bacillus anthracis. Although the incidence of the disease is decreasing in our country, it is still endemic in certain regions of the country. The cutaneous form of the disease is the most common clinical form, which is usually benign and rarely causes bacteriemia and sepsis. In this case report, a cutaneous anthrax case who had positive blood and wound cultures and were complicated with sepsis are presented.

A 39-years-old male patient living in Kars (Eastern Turkey) was admitted with fever, chills, and a wound with swelling redness on the right arm. In his medical history, the patient stated an insect (fly) bite three days ago and consequent development of a lesion on his right arm. He also stated that he had slaughtered a lamb ten days ago by himself. On admission, the patient was detected to have a 2-3 cm centrally necrotic and peripherally edematous wound confined to the right forehand. There was also fever, hyperemia and general edema confined to right arm up to the shoulder level. With the preliminary diagnosis of cutaneous anthrax, the patient was hospitalized, and ampicillin-sulbactam therapy was started, but due to the progression of the lesion and clinical deterioration, the treatment was changed to piperacillin-tazobactam and clindamycin. The swab samples from the wound were sent to the laboratory and revealed Gram-positive sporulated bacilli and following blood cultures were also positive for growth. The agent pathogen was identified as B.anthracis by Gram stains from wound samples and blood cultures which was susceptible to penicillin. MLVA method with 25 loci was used for genotyping, and it was determined that the genotype in our case is GK43 that is located in the major cluster A and subset 3. On the tenth day of hospitalization due to the widespread and necrotic lesions on his arm, compartment syndrome had occurred. Escharatomy had been established for the treatment of comparment syndrome. After three weeks of antibiotherapy, the patient has been discharged from the hospital with good health.

As a conclusion, this case report reminds need of high attention to the clinical course of cutaneous anthrax in order to avoid severe complications such as sepsis.

Key words: Cutaneous anthrax, sepsis, zoonotic infection

Öz

Şarbon, Bacillus anthracis'in etken olduğu zoonotik bir hastalıktır. Ülkemizde hastalığın insidansı genel olarak azalmakla birlikte belirli bölgelerde halen endemik olarak görülmektedir. Hastalığın en sık görülen klinik formu deri şarbonu olup, genellikle selim seyreder, bakteriyemi ve sepsis tablosu nadirdir. Bu çalışmada, yara ve kan kültürlerinden etkenin izole edildiği sepsis ile seyreden bir deri şarbonu olgusu sunulmuştur.

Türkiye'nin Doğu Anadolu bölgesinde yer alan Kars'ta yaşayan 39 yaşındaki bir erkek hasta ateş, üşüme, titreme, sağ kolda yara ve kızarıklık şikayetleriyle başvurdu. Öyküsünde, 3 gün önce sağ kolunu bir böcek (sinek) ısırdığı ve bu ısırık sonrası bir yara geliştiğini bildirdi. Ayrıca hasta bundan on gün önce de bir kuzu kestiğini ifade etti. Hastanın başvuru anında sağ ön kolda 2-3 cm büyüklükte, ortası nekrotik ve ödemli bir lezyon saptandı. Bunun yanında hastanın sağ kolunda ellerden başlayıp omuz seviyesine kadar çıkan ısı artışı, kızarıklık ve ödem mevcuttu. Deri şarbonu ön tanısıyla yatırılan hastaya ampisilin-sulbaktam tedavisi başlandı ancak lezyonlarda kötüleşme ve yayılma olması üzerine tedavisi piperasilin-tazobaktam ve klindamisin olarak değiştirildi. Hastanın lezyonundan gönderilen sürüntü örneklerinin Gram boyasında Gram pozitif sporlu basiller görüldü ve takibinde kan kültürlerinde üreme saptandı. Kan kültürlerinden ve yara sürüntülerinden yapılan Gram boyalarla etken patojen olarak B. anthracis tanımlandı ve yapılan antibiyogramda penisiline duyarlı bulundu. Genotipleme için 25 lokuslu MLVA metodu kullanıldı ve bizim olgumuzdaki genotipin ana küme A ve alt küme 3'te yer alan GK43 olduğu belirlendi. Hospitalizasyonun onuncu gününde kolundaki geniş ve nekrotik lezyonlar nedeniyle hastada kompartman sendromu gelişti; tedavisi amacıyla eskarotomi uygulandı. Üç hafta süren antibiyoterapi ardından hasta şifa ile hastaneden taburcu edildi. Sonuç olarak, deri şarbonu genel olarak antibiyotik tedavisi ile iyileşen bir hastalık olmasına rağmen nadir de olsa sepsisle karakterize mortal bir tabloya dönüşebileceği göz önünde bulundurulmalıdır.

Anahtar sözcükler: Deri şarbonu, sepsis, zoonotik enfeksiyon.

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Introduction

Anthrax is a zoonotic disease that is caused by Bacillus anthracis, which is transmitted to humans directly or indirectly from grass-eating animals [1]. Skin, gastrointestinal and respiratory system anthrax occurs according to the location of entry into the body of the agent. Cutaneous anthrax may develop by cutting of dead sick animals, skin stripping, meat chopping; gastrointestinal system anthrax may develop by eating infected meats, and lung anthrax by inhalation of spores [1, 2]. Most human cases are cutaneous anthrax. Livestock workers, butchers, and veterinarians are occupational groups at risk [1, 2].

Anthrax is an infectious disease whose frequency is gradually decreasing. It is still seen as endemic in our country, and it keeps its importance especially in Central Anatolia and Eastern Anatolia regions. Although the anthrax is still an endemic disease in Turkey; its incidence has been decreased in recent years [3]. The most common type is (%95) cutaneous anthrax [3]. Preventing direct contact with sick animals will significantly reduce transmission. Kars is a region where cattle, sheep and, goat livestock are extensively built and is one of the places where anthrax is still widespread and seasonal outbreaks develop.

This case report is a reminder for clinicians that inadequate treatment and follow-up of cutaneous anthrax can lead to serious complications such as sepsis.

Case report

A 39-year-old male patient living in the village of Tomali, Arpaçay, Kars (Eastern Turkey) was admitted with complaints of fever, chills, redness and wound on the right arm. The patient stated that a fly had bitten fly in his right arm while collecting grass on the field 3 days ago; then he felt burn and itching in the bite area, and after these, an acne-like lesion developed on same area; he also added that he picked up the scar tissue with his hand. Apart from this, the patient said that he had cut a lamb and touched its raw meat ten days before the biting incident. On examination, the patient was conscious, well oriented and cooperative. There were blue-black hemorrhagic bullas (and a necrosis area in the middle of bulla) sized 2x3 cm in three separate sites in the right anterior cruciate area, and there was an increase in temperature, redness, and edema on the right upper extremity from the fingers to the level of the shoulder. In addition, there was a lymphadenopathy at the right axillary region which had a size of 5 cm in diameter. The patient's skin lesion was shown in Figure 1.

On admission, the patient's fever was 39°C, blood pressure was 110/60 mmHg, and pulse was 124 beats/minute. Laboratory values were measured as white blood cells: 13400/microliter (mL), neutrophil ratio: 84%, platelet count: 202.000/mL, mean platelet volume: 7.7 femtolitre (fL), sedimentation rate: 20 mm/s, C-reactive protein: 3 mg/dL. The patient was hospitalized with a diagnosis of complicated cutaneous anthrax. Swab samples from the skin lesion and blood cultures were sent to the microbiology laboratory. The patient was started to be treated with ampicillin-sulbactam 4x1.5 grams (gr) per day intravenously. Daily extremity elevation was provided and extremity dressed with saline. On the second day of treatment, the patient's fever was 41°C, and because of the rapid progression of the patient's lesions on the neck, chest and, back (Figure 2); so the patient was thought to have been into sepsis, and ampicillin-sulbactam was discontinued. Piperacillin-tazobactam 3x4.5 gr/day intravenous and clindamycin 3x900 miligrams (mg)/day intravenous treatment was started.

Blood cultures of the patient were placed on an automated blood culture device, BACTEC (BD, USA), positive signal was obtained following 48 hours of incubation, and Gram-positive bacils were seen in Gram stain (Figure 3); inoculation to 5% sheep blood agar, EMB agar, and chocolate agar medium had been done. Specimens taken from the wound did not show any specific feature in Gram-stain; also no growth was detected in wound cultures. After 24 hours of incubation, in blood cultures, gray colored, small, mat colonies were observed, which did not hemolyze in sheep blood agar. In order to verify and advanced typing, isolated strains were sent to the National Reference Laboratory of Turkey General Directorate of Public Health for high risk pathogens. MLVA method with 25 loci was used for genotyping, and it was determined that the genotype in our case is GK43 that is located in the major cluster A and subset 3. In addition, in our laboratory, the sensitivity test for penicillin was performed with 10 units of penicillin disk (BD, USA) and was found to be sensitive (Figure 4).

On the second day of treatment, respiratory distress developed; and 40 mg methylprednisolone treatment had given for three days. During the hospitalization, the patient continued to have a temperature of 38°C and over. On the eighth day of hospitalization, the general condition was bad; fever was 39oC and blood pressure was 100/60 mmHg. There was widespread bullous lesions in the right arm of the patient and rapid progression in necrotic areas. In the complete blood count, white blood cell, , neutrophil ratio, platelet count and mean platelet volume were 20300/mL, 81.3%, 214.000/mL and 7.9 fL, respectively. On the tenth day of hospitalization, compartment syndrome had been occurred. Escharotomy had been established for the treatment of compartment syndrome. After three weeks of antibiotherapy, the patient has been discharged from the hospital with good health.

A written consent was taken from the patient

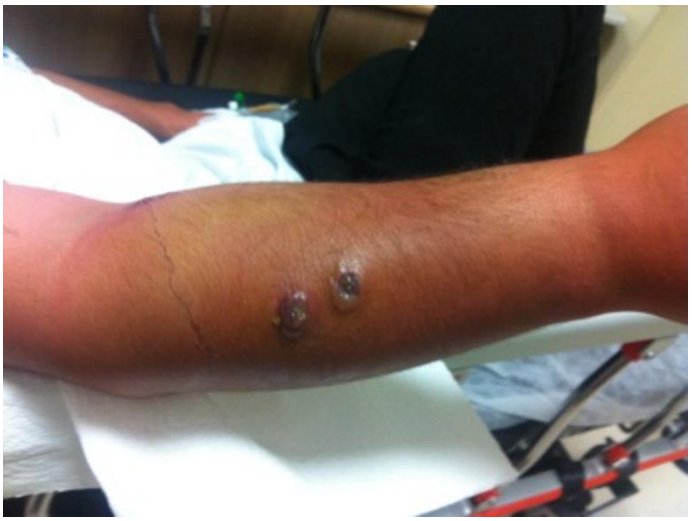

Figure 1. The patient's skin lesion.

Discussion

Although anthrax had been eradicated in developed countries, it is still a major public health problem in developing countries. As a result of the animal vaccination campaigns, education of the people in the risk group and prevention of uncontrolled animal slaughter, the number of cases in our country has decreased considerably. According to the data of the Ministry of Health, the number of human anthrax cases, which was 396 in 2000, dropped to 148 in 2009 and to 93 in 2010. Between the years of 1995-2005, the prevalence of anthrax in provinces of Turkey was given respectively: Kars (477 cases), Ardahan (364 cases), Erzurum (355 cases) and Van (351 cases) [3].

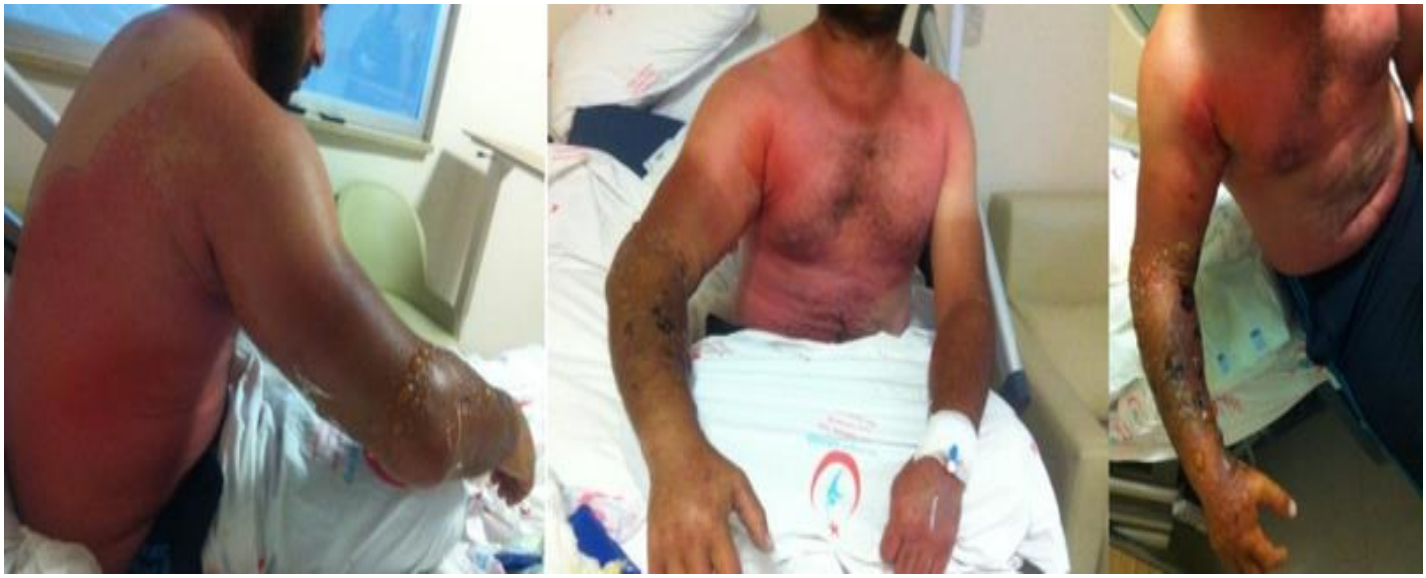

Figure 2. Patient's lesions on the second day of the treatment.

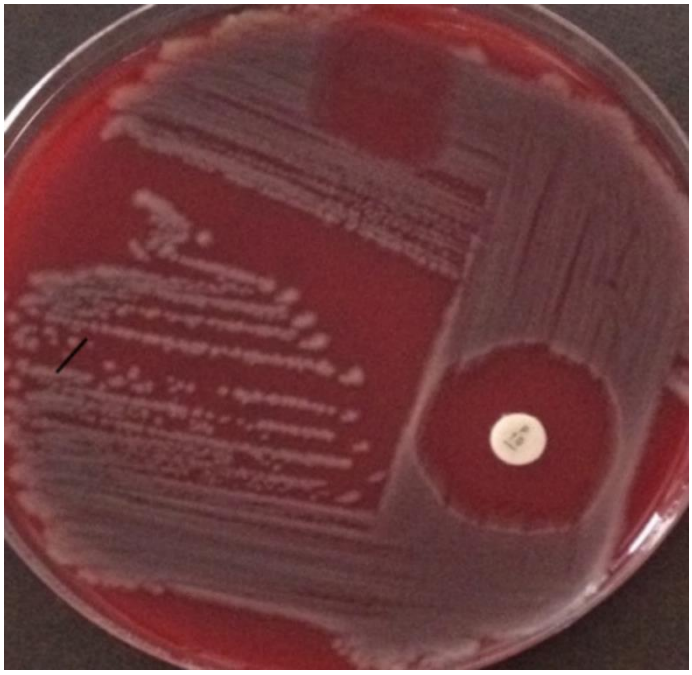

Figure 3. Penicillin sensitivity of obtained Anthrax isolate.

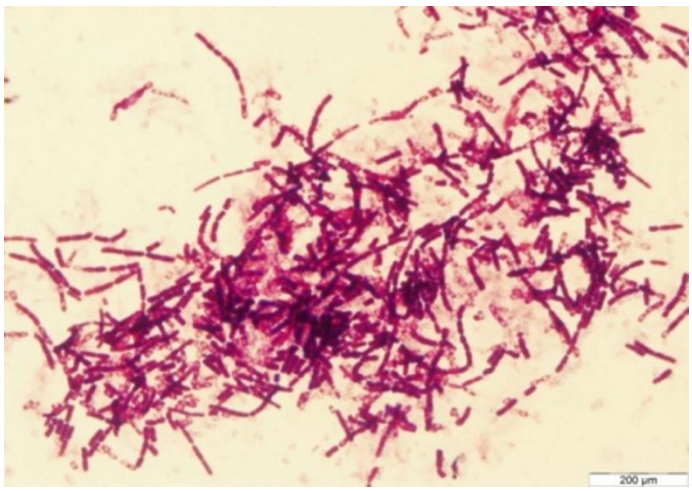

Figure 4. Gram stain from blood culture.

The skin anthrax is the most common (95%) clinical form of the disease. Often the anthrax is picked up from a body region where the integrity of the skin is corrupted by direct contact during the cutting or skinning of the sick animals or chopping the meat. There is a study claiming that anthrax can also be transmitted through the bite of a mosquito. Anthrax lesions are most commonly seen in open areas of the body such as the hand, arm, neck, nape, and face [5-8]. Similarly, in our case, the anthrax lesion developed in an open area of the patients' arm which was bitten by a mosquito and was contacted to raw meat of a lamb ten days ago. Our patient stated that he could not make an anthrax vaccination on some of his animals, and he also added that veterinary control was missing. This has shown that the controls made during animal production can sometimes be neglected; and that in addition to controls, animal husbandry people should be well aware of the disease to be able to prevent anthrax disease in our region completely.

Although the development of sepsis in the cutaneous anthrax is very rare; it is the most important complication in this clinical form [5-7]. In the study of Doganay et al. [5], 22 skin cancer cases followed up for seven years: Septic shock and bacteriemia were seen in two patients, while 10 of 22 cases showed serious infection signs. In a study of 58 cases of skin eczema in our country, only one case was developed as clinical and laboratory sepsis [6]. No sepsis-like complication has been reported in the anthrax case series in Eastern Anatolia and the Marmara region [9, 10].

Skin anthrax can usually heal spontaneously and 3-7 days of treatment is considered to be sufficient for uncomplicated cases [1, 2]. As in our case, 10-14 days of treatment is recommended for complicated cases with sepsis [1]. 10-20% of untreated cases develop sepsis and mostly result in death. However, close follow-up and appropriate treatment have reduced mortality to about 3% [1, 2]. Demirdağ et al. [7] reported a mortality rate of 8% due to sepsis in skin anthrax cases. Kaya et al. [11] In a study evaluating 132 patients in the Eastern Anatolia region, the death rate was 1.5% due to malignant edema and meningitis. High mortality rates in systemic anthrax infection are associated with lung anthrax and anthrax meningitis [1, 2, 12].

Although the lesion is easily characterized by its typical appearance, the lesion is confirmed by the appearance of gram-positive capsular bacilli in the gram stained preparation made

from clinical specimens and by growth of the agent [1, 2]. Encapsulated gram-positive bacilli were observed in the gram staining of our case's bullous lesions, and the anthrax was isolated from wound and blood cultures. The antimicrobial susceptibility of the isolates which confirmed by conventional and molecular methods was investigated by epsilometer-test. The strains were found sensitive to ampicillin, tetracycline, tigecycline, ciprofloxacin, levofloxacin, gentamycin, chloramphenicol, erythromycin, clarithromycin, vancomycin, linezolid, daptomycin, and rifampicin. These results are consistent with the results of sensitivity studies abroad and in our country [13-16]. This data shows that there is no resistance problem in naturally occurring anthrax disease and that penicillin, quinolone, and doxycycline are still the first antibiotics to be preferred in treatment.

Comparing the first and last complete blood counts from the patient, leukocytosis seems to be exacerbated with time; this increase is normalized in cases where the general situation is gradually deteriorating, and the infection cannot be controlled. Again, the patient's last mean platelet volume value is higher than the first one. This increase in mean platelet volume is consistent with publications that indicate that it is increasing in infective conditions [17].

Molecular typing methods are needed to be determined the epidemiological relationship between the cases. Molecular typing can establish epidemiological relationships between different patients and environment and / or animal isolates; genotypes predominant in a geographical area can be identified; obtain information about the origin of the species in the country and the continuity of the dominant clones [4, 18-20]. In this study, MLVA method with 25 loci was used, and it was determined that the genotype in our case is located in the major cluster A which is widespread throughout the world. According to MLVA results with eight loci, B.anthracis genotypes are divided into two strains (A and B) containing six (A1-4, B1-2) branches. While cluster A has spread throughout the world, cluster B has a more limited geographical area (B1 is dominant in South Africa, and B2 is in Europe). The subset A1 in cluster A is distributed worldwide but is more dominant in the western part of North America. While the A3 cluster contains dominant genotypes observed in many parts of the world, the A4 subset includes genotypes scattered in Asia, Europe and America [19]. A3a (which is a subset of A3 subset) is observed in the Georgia, Turkey and Iran, and southern and eastern Bulgaria [4, 18-20]. When genotyping results of B.anthracis strains in our country are examined; the most common genotypes are GK 35, GK 43 and GK 44 in the subgroup A3.a [4,18]. Our isolate was also typed as GK 43 and was found to be similar to other strains isolated from Konya, Ankara, Kayseri, Eskişehir, and Erzurum. When the data obtained with MLVA-25 are examined, the presence of strains isolated in Kars, Erzurum, Kayseri, Ankara, Konya and Eskişehir in the same cluster between 2004 and 2012 indicates that the infection did not occur within a specific province or a limited period. This data demonstrates that the cross contamination rate between human and animal anthrax cases in our country is quite high.

Anthrax disease is seen as sporadic in our country and surrounding countries. The development of sepsis in the patient with the diagnosis of skin anthrax and the isolation of anthrax bacillus in the blood culture will stimulate the physicians who may encounter similar cases. In conclusion, we believe that patients with contact with livestock and who thought to have skin anthrax may have been treated with early and appropriate treatment in terms of sepsis development and systemic complications.

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