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Serum levels of anti-carbonic anhydrase antibodies and erythrocyte oxidative stress markers in endometriosis

Ulku Mete Ural¹ · Ahmet Alver² · Hasan Efe³ · Ayse Senturk² · Imran Ince² · Beril Gurlek¹

¹Department of Obstetrics & Gynecology, Recep Tayyip Erdogan University, Faculty of Medicine, Rize, Turkey

²Department of Biochemistry, Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey

³Department of Biochemistry, Recep Tayyip Erdogan University, Faculty of Medicine, Rize, Turkey

ABSTRACT

Aim: To evaluate the serum levels of anti-carbonic anhydrase I-II antibodies and erythrocyte oxidative stress markers in endometriosis.

Method: This case-control laboratory investigation was performed in the obstetrics and gynecology department of a tertiary center. Serum anti-carbonic anhydrase I and II antibodies and erythrocyte oxidative stress markers (superoxide dismutase, malondialdehyde, glutathione peroxidase and catalase) were compared with control group (n = 30) in the endometriosis group (n = 33). Correlation between carbonic anhydrase autoantibodies and oxidative stress markers were tested.

Results: Serum levels of anti-carbonic anhydrase II antibodies were found to be significantly increased in the endometriosis group compared to controls. The erythrocyte antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase and catalase) and malondialdehyde levels in erythrocytes were increased in endometriosis group; but only glutathione peroxidase activity and malondialdehyde levels were significantly higher in endometriosis group. No correlation was detected between anti-carbonic anhydrase antibodies and oxidative stress markers.

Conclusions: Our results indicate that erythrocyte oxidative stress and anti-carbonic anhydrase antibodies may be involved in the pathophysiology of endometriosis.

Keywords: Endometriosis, carbonic anhydrase I, carbonic anhydrase II, antibodies, antioxidants.

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✉ Dr. Ulku Mete Ural,

Bolu Abant İzzet Baysal University, School of Medicine

Department of Obstetrics & Gynecology, Bolu, Turkey

E-mail: ulkumete2004@yahoo.com

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Introduction

Endometriosis is the ectopic growth of endometrial cells. It can be seen in about 10% of women of reproductive age [1]. The etiology

of endometriosis has not been clarified yet. However, oxidative stress and malfunction of cellular and humoral immunity are possible mechanisms associated with the pathogenesis [2,3]. Endometriosis patients have diminished concentrations of antioxidant enzymes reminding that reduced peritoneal antioxidant levels may reflect an increase in the levels of reactive oxygen species (ROS) levels [2]. Recently, biochemical changes like oxidation

of membranes and glutathione decrement have been reported in erythrocyte [4].

Antibodies directed against endometrial antigens and accumulation of complement system have been demonstrated in endometriosis patients. Antigens triggering such an immune response may develop against the human chorionic gonadotropin receptor, including the enzymes carbonic anhydrase (CA) I and II, CA 125 and transferrin in endometriosis [3,5].

Carbonic anhydrases (CA; EC 4.2.1.1) activate the transformation of carbon dioxide and the bicarbonate ion. In mammals, sixteen isoenzymes of CA have been described and those have different tissue distribution and cellular localization. They participate in important metabolic reactions including respiration, transport of CO₂ and bicarbonate, maintenance of carbon dioxide balance and pH, and excretion of electrolytes [6]. Even though CA I and CA II exist in the epithelium of various organs, they are mainly expressed in erythrocyte. CA I activity is has a remarkably reduced action than CA II and thus, it is important the majority of the total CA activity. Recently, several publications demonstrated the pattern of autoimmune reaction against CA II in various pathologies [7-10]. The mechanism causing antibody creation has not been yet elucidated but oxidative stress is supposed to be involved in this etiopathogenesis [11].

In recent years, CA autoantibodies have been determined in some autoimmune diseases. To the best of our knowledge, the association between anti-CA antibodies (Anti-CAA) with antioxidant enzymes in endometriosis has not yet been tested in the medical literature (PubMed). Our aim was to analyze the levels of erythrocyte oxidative stress markers and anti-CAA in endometriosis, and to seek whether there is a relationship between these indicators

and discuss a possible role of anti-CAA in the pathogenesis of endometriosis.

Materials and Methods

This case-control laboratory investigation was done in the Obstetrics and Gynecology Department of a University Hospital after the approval of the local Institutional Review Board (Date and Decision no: 2014/04/17, 2014/65). The rights of all participants were protected and written informed consents were obtained before the study according to the Helsinki Declaration. This study included 33 women with endometriosis and 30 healthy women. The diagnosis of all patients with endometriosis was confirmed histologically after laparoscopy. Patients in the endometriosis group had at least stage 3 endometriosis. Control groups consisted of age-matched healthy, regularly menstruating women without pain, pelvic anomalies and previous history of pelvic surgery. In the past, they had at least one successful pregnancy.

Exclusion criteria consisted of a history of pregnancy loss, systemic diseases, smoking habit, use of any medications [such as oral contraceptives, hormonal drugs, steroids, aspirin] in the last 3 months and unwillingness for enrollment in the study. Serum levels of anti-carbonic anhydrase I and II antibodies and erythrocyte oxidative stress markers (superoxide dismutase, malondialdehyde, glutathione peroxidase, and catalase) were compared and the correlation between these indicators were tested.

Measurement of serum Anti-CAA I and Anti-CAA II: Human erythrocyte CA I and CA II isoenzymes were purchased (*Sigma Chemical Co., St. Louis, MO, USA*). Serum anti-CAA II titers were determined by ELISA method as described in previous publication [8]. Briefly,

ELISA plates (BD Biosciences, USA) were coated with CA-I and CA-II (50 μ L of 10 μ g/ml) in carbonate buffer (0.05 mM, pH=9.6) and incubated overnight at 4°C. The coated plates were washed phosphate buffer (pH=7). Skimmed milk (2%) in phosphate buffer was used for blocking. After washing process, incubation of the wells with 100 μ L of serum diluted with dilution buffer was carried out (1:200). Following washing, every well was incubated with 100 μ L of 1:2000 dilution of peroxidase-conjugated anti-human IgG antiserum (*Sigma Chemical, St. Louis, MO, USA*). After the final washing, ELISA wells were incubated with 100 μ L o-phenylenediamine solution and this reaction was quit by 100 μ L of 2 M H₂SO₄. The absorbance of wells were read at 480 nm. The assays were performed twice and specific binding of serum antibody to anti-CAA I or anti-CAA II was determined by subtraction of the average absorbance of control wells (uncoated with CA) from that of antigen coated wells.

Determination of malondialdehyde (MDA) levels in erythrocytes: The MDA levels in erythrocytes were determined as described in a previous publication (*Agilent 1100 series HPLC systems, Waldbronn, Germany*), C18 column was used for measurements (*ZORBAX Eclipse XDB-C18; 4.6 x 150mm; Agilent Technologies, Santa Clara, CA, USA*) [12]. Detection was performed fluorometrically (excitation 536 nm and emission 555 nm). Tetraethoxypropane was utilized as MDA standard (1.25–0.035 Mm) and results were represented as mM / g Hb.

Determination of catalase (CAT) activity: The catalase activity in erythrocytes was determined by the method as described in relevant literature

[13]. This method relies on the fact that the absorbance at 240 nm is diminished due to the dismutation of H₂O₂ and results are demonstrated as k/ g Hb (k, rate constant)

Determination of superoxide dismutase (SOD)

activity: The SOD activity in erythrocytes was assessed by the method as described in a previous publication [14]. Formazan formation was evaluated spectrophotometrically at 560 nm and results were shown as U/g Hb.

Determination of glutathione peroxidase (GPx)

activity: The activity of GPx was measured with a spectrophotometric assay kit (*Assay Desings, Ann Arbor, MI, USA*). In this kit, the results were expressed as U/g Hb.

Statistical analysis

Data derived from the results of this study was analyzed using IBM SPSS Statistics 20 program. The normal distribution of data was assessed using Kolmogorov-Smirnov test. A comparative analysis of the erythrocyte superoxide dismutase, catalase, and glutathione peroxidase and malondialdehyde levels was carried out via the independent samples T-test. Mann-Whitney U was employed for the other non-parametric comparisons. Correlation between variables was tested using Pearson's correlation test. The level of significance was set at p value < 0.05.

Results

As indicated in Figure 1 and Table 1, serum anti-CAA I titers did not differ between endometriosis and control groups (0.39 ± 0.12 vs. 0.37 ± 0.08 ; respectively, $p=0.41$). On the other hand, anti-CAA II titers were remarkably increased in endometriosis patients than that in controls (0.30 ± 0.11 vs. 0.241 ± 0.080 ; $p=0.027$) (Table 1). The absorbance values > 0.401, the

mean absorbance ± 2 SD of control subjects, were termed as positive. The positive results were noted in 7 of 33 endometriosis cases (Figure 2).

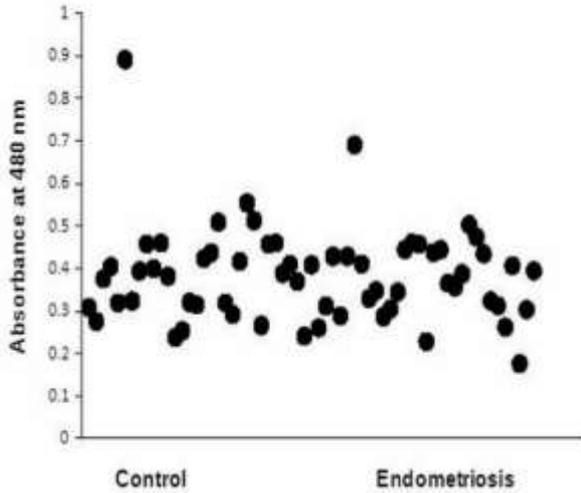


Figure 1. Anti CAA I in sera from endometriosis patients and control subjects.

Table 1. Serum levels of oxidative stress markers and anti-carbonic anhydrase antibodies in control and endometriosis groups.

Variable	Groups		p Value
	Control	Endometriosis	
Anti-CAA I (absorbance)	0.392 \pm 0.12	0.378 \pm 0.98	0.41
Anti-CAA II (absorbance)	0.241 \pm 0.08	0.300 \pm 0.11	0.027*
SOD (U/g Hb)	50.99 \pm 18.99	62.43 \pm 32.51	0.29
CAT (k/g Hb)	68.51 \pm 39.52	80.85 \pm 39.31	0.24
GPx (U/g Hb)	1.50 \pm 0.38	1.67 \pm 0.24	0.038*
MDA (mM/g Hb)	5.50 \pm 0.68	6.03 \pm 1.14	0.037*

Values: Mean \pm SD; Anti-CAA= Anti-carbonic anhydrase antibodies; SOD= Superoxide dismutase; CAT= Catalase; GPx= Glutathione peroxidase; MDA= Malondialdehyde; *= statistically significant.

The antioxidant enzyme activities (SOD, CAT and GPx) and MDA levels in erythrocytes were

increased in endometriosis group; but only GPx activity and MDA levels were significantly higher in endometriosis group ($p=0.038$, and $p=0.037$, respectively) (Table 1). As shown in Table 2, no correlation was detected between anti-CAA I and II titers and SOD, CAT and GPx activities and MDA levels.

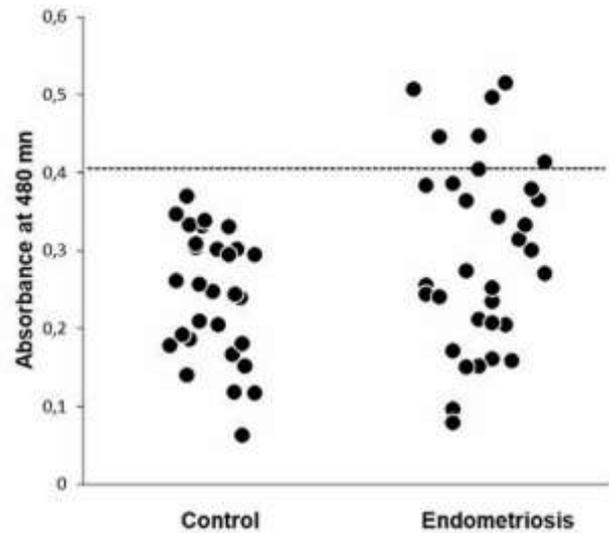


Figure 2. Anti-CAA II in sera from endometriosis patients and control subjects. The line indicates the mean value ± 2 SD of control subjects (A480 = 0.401).

Table 2. Correlation analysis of anti-carbonic anhydrase antibodies to oxidative stress markers in the endometriosis patients.

Variable		r Value	p Value
Anti-CAA I	SOD	-0.205	0.148
	CAT	-0.072	0.346
	GPx	0.030	0.435
	MDA	-0.127	0.244
Anti-CAA II	SOD	-0.006	0.488
	CAT	0.163	0.183
	GPx	-0.209	0.122
	MDA	0.037	0.426

Anti-CAA= Anti-carbonic anhydrase antibodies; SOD= Superoxide dismutase; CAT= Catalase; GPx= Glutathione peroxidase; MDA= Malondialdehyde; *= statistically significant.

Discussion

The present study was performed to assess endometriosis patients in terms of serum levels of anti-CAA I, anti-CAA II and erythrocyte oxidative stress markers. Our results have shown that in subjects with endometriosis anti-CAA II frequency [21%] and erythrocytes oxidative stress markers were higher than control subjects (Figure 2 and Table 1). We concluded that there is an increased immune response to CA II in endometriosis patients. Increased erythrocyte oxidative stress observed in endometriosis may be effective in the mechanism of CA II autoantibody. These findings were similar to previous results reported in patients with polycystic ovary syndrome (26%), systemic lupus erythematosus (31.2%), Sjögren's Syndrome (17%), and rheumatoid arthritis (27.8%) [7,8,15,16].

Endometriosis was encountered in approximately 20-50% of infertile women that underwent laparoscopy during their infertility workup [2]. Since alteration of humoral and cellular immune systems is a feature of endometriosis, new diagnostic measures have focused on immunologic parameters related to endometriosis [17]. Elevated levels of ROS were previously reported to be associated with endometriosis [18,19]. Stimulation and improvement of mononuclear phagocytes lead to oxidative stress which causes a localized pelvic inflammatory process [20]. Szczepańska et al. reported a noteworthy reduction of SOD and GPx activities in peritoneal cavity with endometriosis [21]. In addition, it has been reported that oxidative stress observed in endometriosis also affects red blood cells [4].

Our results indicate that erythrocyte GPx activity and MDA levels were significantly higher in endometriosis patients compared to controls. It must be remembered that there is a critical interaction between oxidative stress and

cytokines, chemokines and antioxidants. The levels of all of the antioxidants may not be necessarily altered significantly in order to eliminate the hazardous effects of oxidative stress. In addition, their levels may not always reflect the changes in the microenvironment influenced directly by the pathology. All in all, whether and to what extent oxidative stress is involved in endometriosis remain to be elucidated in further trials.

Carbonic anhydrases possess a serious function in gas transportation, acid-base balance and multiple secretion tasks in tissues [22]. The endometrium and placenta are well-known sources of CA-I and CA-II suggesting a possible role of these enzymes in fertilization and fetoplacental development [23]. Since CA I and CA II are the most frequent human isoenzymes, immunoglobulin G autoantibodies formed against CA I and CA II have been identified in various autoimmune diseases [15,16,24,25]. In our study, serum levels of only anti-CAA II were significantly increased in women with endometriosis.

The way of antibody formation against CA II has not yet been clearly elucidated, however, protein modifications and oxidative imbalance are important reasons for autoimmunity. Fuji et al. has reported that elevated oxidative stress in erythrocytes causes antibody production against CA II [11]. The final products of lipid peroxidation such as HNE (4-hydroxy-2-nonenal) and MDA bind to proteins and change their antigenic features. One of the major targets of these end-products in red blood cells is CA II [26]. In case of erythrocytes in endometriosis are prone to oxidative stress, CA II may gain an antigenic character as a result of similar alterations. In an experimental study, anti-CAA II was found to inhibit the enzymatic activity of CA [27].

From our results, we understood that anti-CAA II, rather than anti-CAA I, seem to be involved in endometriosis. Identification and discrimination of CA I and CA II in terms of functional and physiological impacts can provide a better understanding. We found no correlation between oxidative stress markers and autoantibodies against carbonic anhydrase enzymes. The complex interaction between immune system and antioxidant mechanisms constitute a challenge for researchers to make more accurate and precise interpretations. Further attempts must be directed to explore the functions of subgroups of antibodies and antioxidant systems individually.

Conclusion

Erythrocyte oxidative stress and anti-CAA II may be associated with the pathophysiology of endometriosis. Taking the complexity of inflammatory reactions into account, the beneficial effects of oxidative stress balance and immunomodulation need to be investigated further using larger study groups.

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ORCID iD of the author(s)

Ulku Mete Ural / 0000-0002-2176-7863

Ahmet Alver / 0000-0002-9617-6689

Ayşe Senturk / 0000-0002-3542-2153

Imran Ince / 0000-0003-2232-3444

Beril Gurlek / 0000-0002-4050-3193

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Evaluation of renoprotective effect of calcium channel blockers in coronary angiography patients



Burçin Meryem Atak Tel · Memnune Sena Ulu · Sinan Kazan · Mehmet Polat

Department of Internal Medicine, Afyon Kocatepe University, Faculty of Medicine, Afyon, Turkey

ABSTRACT

Aim: To evaluate the effectiveness of contrast-induced acute kidney injury (CI-AKI) prophylaxis retrospectively, using calcium channel blockers (CCB) before and after contrast exposure and comparing them with patients using angiotensin converting enzyme inhibitors (ACEI), which has not been explored by many studies.

Methods: The study was performed in Afyon Kocatepe University, Faculty of Medicine Research Hospital, Cardiology Department between January 2014 and June 2016. Eighty patients using dihydropyridine (amlodipine 10 mg), non-dihydropyridine (diltiazem 60 mg) CCB or ACEI in the form of monotherapy before coronary angiography were included.

Results: In the CCB and ACEI group, CI-AKI development rates were 15.7% (n=8) and 24.1% (n=7), respectively ($p = 0.383$; Fisher's exact test). When the CCB group was evaluated as dihydropyridine and non-dihydropyridine subsets, CI-AKI development rates were found to be similar as well ($p = 0.445$; Fisher's exact test) in each subset.

Conclusion: In our study, we evaluated one of today's important dilemma; the methods related to the prophylaxis of CI-AKI. Our study shows that there is no difference in the development of CI-AKI between patients using the calcium channel blocker group drugs and ACEI as monotherapy. However, in our study, the mean age of patients using CCB was significantly higher than the group using ACEI.

Keywords: Contrast-induced acute kidney injury, calcium channel blockers, angiotensin converting enzyme inhibitors, renoprotective effect.

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✉ Dr. Burçin Meryem Atak Tel

Department of Internal Medicine, Afyon Kocatepe University, Faculty of Medicine, Afyon, Turkey

E mail: burcinatak@hotmail.com

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Introduction

Contrast media (CM) has a plethora of applications in routine non-invasive or percutaneous invasive imaging examinations

and therapeutic interventions. Unfortunately, the use of CM is associated with a number of complications, the most serious being contrast-induced acute kidney injury (CI-AKI) [1].

A general definition of CI-AKI is an impairment in renal function occurring within 3 days following the intravascular administration of CM and the absence of an alternative etiology [2]. Contrast media administration has been said to be the third leading cause of

hospital-acquired acute renal failure in the past 3 decades [3]. Chronic kidney disease, dehydration, diabetes mellitus (DM), advanced age, increased volume of CM and recurrent administrations are well-known risk factors of CI-AKI [4].

In spite of the vast clinical importance of CI-AKI, its understanding and the pathophysiology behind CI-AKI is not fully explained [2, 3]. Most reviews show a complex pathophysiology overlaying medullary ischemia and hypoxia, oxidant damage, intratubular obstruction, hypertonicity, plasma viscosity and many pathways including endothelins, nitric oxide, reactive oxygen species, prostaglandins and adenosine [5].

Calcium has been proposed as a mediator of the vasoconstrictor response to CM [6]. Also Intracellular Ca^{2+} overload is considered to be a key factor in CI-AKI [3]. The rationale is based on the fact that while in normal subjects, the Na^+ - Ca^{2+} exchanger pumps Ca^{2+} outside the renal tubular epithelial cells to keep intracellular Ca^{2+} low. Under the effect of CM, the Na^+ - Ca^{2+} exchanger can reversibly extrude Na^+ for Ca^{2+} influx, thereby leading to intracellular Ca^{2+} overload, which is considered a key factor in ischemic cell injury and in CI-AKI [7]. The increase in intracellular calcium provokes a vasoconstrictive response in intrarenal circulation and would be an important mediator of epithelial cell apoptosis and necrosis. Thus, calcium channel blockers (CCB) have been hypothesized to have protective effects against CI-AKI [8]. The CCB attenuated the vasoconstrictor response of CM in animal studies, although prophylactic use of CCB has not gained wide acceptance [6]. Literature show opposing results; some authors suggesting them to be protective [9, 10], others finding no benefit at all [11-13]. However, these researches are very old and more recent

studies are needed. In this study we compared patients who used dihydropyridine (amlodipine 10 mg), nondihydropyridine (diltiazem 60 mg) CCB and ACEI; and underwent coronary angiography. We compared serum creatinine, blood urea nitrogen (BUN), urea levels and glomerular filtration rates (GFR) before and 3-15 days after the coronary angiography. We planned to examine the changes in the GFR values by taking demographic data into consideration.

Materials and methods

We used a definition of CI-AKI which is widely accepted; impairment in renal function occurring within 3 days following the intravascular administration of CM and the absence of an alternative etiology [2].

The study included 80 patients using dihydropyridine, non-dihydropyridine CCB and ACEI from 4027 patients who underwent coronary angiography in the Cardiology department of Afyon Kocatepe University, Faculty of Medicine, Research Hospital between January 2014 and June 2016. Information about patients was obtained by retrospectively examining patient files. Prior to the study, the necessary ethics committee approval was obtained (Decision no; 29-5-2016). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Inclusion and exclusion criteria: Of the 4027 patients; only 80 patients used dihydropyridine (amlodipine 10mg), nondihydropyridine (diltiazem 60mg) or ACEI before coronary angiography as monotherapy. 2635 patients using multidrug therapies were excluded. Also

1074 patients were excluded because they were not using CCB or ACEI.

Individuals under the age of 18 and over 85 were not included in the study (total of 84 patients). Data was obtained by retrospectively scanning patient files. Patients were included in the study if the registration data were sufficient. Patients were excluded if serum creatinine, BUN, urea levels and GFR value within 3 to 15 days of coronary angiography were not in the database. Also patients with missing demographic data were excluded. 154 patients were excluded because of a missing data in their files.

Statistical Analysis

The data of the patients' who are included in the study has been collected and submitted in a database for the study. These variables include; age, gender, smoking habits, body mass index (BMI), hypertension duration (in years), systolic and diastolic blood pressure measurements, comorbid disease, patients laboratory findings (before and after contrast exposure) including hemogram, BUN, serum creatinine, urea, glomerular filtration rate (GFR), lipid analysis, and electrolytes. Data was analyzed using IBM SPSS 18.0. Descriptive statistical results of the study data were expressed as arithmetic mean \pm standard deviation. While evaluating the groups, patient distributions were given as frequencies. The data obtained were evaluated primarily with descriptive statistics. The suitability of the data to the parametric conditions was evaluated with the Kolmogorov-Smirnov test. Mann-Whitney U test was used for the quantitative evaluation between the CCB and ACEI groups. Wilcoxon Test was used to compare the median values of the two dependent groups, and the Chi Square test was used to compare the categorical data and the groups. In the results obtained from the

statistical tests applied, it was considered significant when a 95% confidence interval (CI) and the *p-value* below 0.05.

Results

When the demographic data of the patients included in the study were evaluated, 55 (68.8%) were male and 25 (31.2%) were female. There were 29 (56%) men, 22 (44%) women in the patient group using calcium channel blocker; and 26 (89%) men and 3 (11%) women in the ACEI group. There was a significant difference in gender distribution between the two groups ($p = 0.002$). The mean age of the patients was 60.4 ± 12.5 . When analyzed as CCB and ACEI groups, the average age of patients using CCB was 62.6 ± 12.6 , and the average age of the patient group using ACEI was 56.5 ± 11.5 . The mean ages of the two groups were found to be statistically different ($p = 0.01$). BMI was similar between the CCB group and the ACEI group ($p = 0.222$). When systolic blood pressures of patients were evaluated, mean systolic blood pressure was 132.9 ± 15.3 (range: 110-180) mmHg in the CCB group and 119.1 ± 10.6 (range: 86-140) mmHg in the group using ACEI. Systolic blood pressure was significantly higher in the CCB group than the ACEI group ($p < 0.001$). Again, when groups using CCB and ACEI were compared, diastolic blood pressures were similar ($p = 0.663$). The duration of involved drug use in both groups was similar ($p < 0.233$). Of the 80 patients who are enrolled in the study, 25 had no other comorbid disease. 31 (38, 8%) had DM, 5 (6.3%) had a history of cerebrovascular disease, 7 (8.8%) had congestive heart failure, 3 (3.8%) had peripheral arterial disease, 15 (18.8%) had hyperlipidemia, 10 (12.5%) had chronic lung disease. Disease distributions were similar in CCB and ACEI groups ($p > 0.5$).

When the drugs used by patients were classified, 21 people were using nondihydropyridine (diltiazem 60mg), 30 people were using dihydropyridine (amlodipine 10 mg) and 29 people were using ACEI.

When staged according to the JNC8 report, only 10 of the 80 patients were found to have optimal systolic and diastolic blood pressure. Despite the use of CCB, blood pressure levels of 5 patients were found to be stage-2 hypertension. It was found that patients using trandolapril had more effective control over blood pressure compared to patients using CCB.

The laboratory values of the two groups are presented in Table 1. When the laboratory values of the two groups were compared, the hemoglobin, hematocrit, platelet, fasting blood glucose, uric acid, calcium (Ca), AST, ALT, lipid panel (LDL, VLDL, HDL, total cholesterol, triglyceride) values were also similar between the CCB group and the ACEI group. There was a significant difference between phosphorus and HbA1c levels between the two groups. Serum phosphorus level in the CCB group was 2.6-4.5 (mean 3.51 ± 0.5) mg / dl, and in the group using ACEI 1.5-4.2 (mean 3.1 ± 0.6) mg/dl ($p = 0.043$). The HbA1c level

Table 1. Comparison of laboratory parameters of two groups.

Laboratory Parameters	CCB Group		ACEI Group		<i>p-value</i>
	Min-max	Mean	Min-max	Mean	
Hb(g/dl)	9- 17	12.7±2.0	9,6-17,0	13.07±2.1	0.538
Htc (%)	28-57	40.3±6.5	29-50	40.6±6.1	0.641
Plt (x10 ³)	66-369	232.3±64.3	177-352	239.4±55	0.944
FBG (mg/dl)	60-377	142.6±65,9	74-358	158.5±76.5	0.597
Uric Acid (mg/dl)	2,8-27	5.8±3.5	2.8-12	5.6±2	0.768
Ca ⁺⁺ (mg/dl)	7,9-10	9.1±8.7	7,8-10	9.1±0.5	0.864
Phosphorus (mg/dl)	2,6-4,5	3.51±0.5	1,5-4,2	3.1±0.6	0.043
K ⁺ (mEq/l)	3,1-5,5	4.4±0.5	3,2-5,1	4.4±0.4	0.028
HbA1c %	5,3-12	7.8±1.8	9,0-13,0	10.4±1.6	0.017
AST (U/L)	13-78	26.7±12.5	12-206	40.4±38.7	0.222
ALT (U/L)	5-167	28.8±31.4	8-61	22.8±12.08	0.980
LDL (mg/dl)	25-228	114.2±46.3	51-212	108.5±40.9	0.572
VLDL (mg/dl)	9-106	31.8±17.5	9-63	29.2±14.2	0.663
HDL (mg/dl)	14-68	37.7±12.4	16-61	36.4±10.1	0.676
Total cholesterol(mg/dl)	120-324	168.1±58.8	84-284	161.1±48.6	0.420
Triglyceride(mg/dl)	85-531	151.4±94.0	48-316	147.5±72.3	0.890

CCB: calcium channel blockers, ACEI: angiotensin converting enzyme inhibitors Hb: Hemoglobin, Htc: hematocrit, Plt: Platelet. FBG: fasting blood glucose.

was 5.3-12% (mean: 7.8 ± 1.8) in the CCB group and 9-13% (10.4 ± 1.6) in the group using ACEI ($p = 0.017$). Serum potassium level was 4.2 ± 0.5 mEq/dl in the CCB group, and 4.4 ± 0.4 mEq/dl in the ACEI group. There was a significant difference between the potassium levels of two groups ($p = 0.02$).

Pre-contrast exposure serum creatinine, BUN, urea and GFR were similar (p values = 0.359, 0.904, 0.707, 0.426, respectively). The mean urea of the group using CCB after contrast exposure was 43.1 ± 23.0 mg/dl, and 38.9 ± 23.6 mg/dl in the ACEI group ($p = 0.08$). Cr value of CCB group was 1.06 ± 1.4 mg/dl, and 1.0 ± 0.4 mg/dl in the ACEI group ($p = 0.11$). After contrast exposure, GFR was calculated as 85.9 ± 27.9 (ml/min/1.73m²) for the CCB group and 88.9 ± 33.9 (ml/min/1.73m²) for the ACEI group. When the two groups were compared in terms of GFR levels after contrast exposure, they were found similar ($p = 0.818$).

In the CCB and ACEI groups, CI-AKI development rates were 15.7% (n=8) and 24.1% (n= 7), respectively. The two groups were similar in terms of CI-AKI development rates ($p = 0.383$; Fisher's exact test).

When the CCB group was evaluated as dihydropyridine and nondihydropyridine groups, the rates of CI-AKI were similar ($p = 0.445$; Fisher's exact test).

Discussion

CI-AKI has become an important problem as a result of the increased use of contrast today. Again, it increases the life-threatening complications such as sepsis, bleeding, and respiratory failure, and increases the hospital stay and leads to an increase in medical costs. Prevention and treatment of such an important complication is very essential for the physician, patient and the country's economy. The data obtained up to this day confirms the idea that

the volume expansion method is the most important method for reducing the risk of CI-AKI [14]. But the pathophysiology behind CI-AKI is not fully explained [2, 3]. Therefore search for a prophylaxis of CI-AKI still continues.

Intracellular Ca²⁺ overload is an important factor in ischemic cell injury and considered to be a key factor in CI-AKI pathophysiology [3]. Therefore CCB, which could prevent intracellular Ca²⁺ overload, have been suggested as a protective measure to prevent CI-AKI [7]. However previous data shows conflicting results. Most of the animal studies on rats showed promising results. Yu-Yan Fan et al., Aritomi et al. and Duan et al. all showed similar renoprotective effects of CCB [15-17]. Beyazal et al. [14] compared isotonic sodium chloride infusion alone, 5% dextrose solution with sodium bicarbonate infusion and isotonic sodium chloride infusion plus 3 days of CCB therapy (one day before and two days after the contrast exposure) for CI-AKI prophylaxis. They find no significant difference between groups.

Arici et al. [18] also find no significant difference in a prospective study with patients pretreated with amlodipine; a dihydropyridine CCB; than placebo. Whereas Russo et al. [10] reported that CCB nifedipine may prevent AKI induced by hyperosmolar contrast agent. Neumayer et al. [9] investigated a total of 35 patients after intravascular administration of contrast media to determine the effects on renal function of a 3-day treatment with the CCB nitrendipine (n=16), compared the findings in a placebo-treated control group (n=19). Prophylactic use of nitrendipine preserved the glomerular filtration rate, whereas control patients showed a significant (27%) reduction in GFR two days after contrast-media injection ($p \leq 0.01$). As a result, it was emphasized that

nitrendipine, a nondihydropyridine CCB, could decrease the risk of CI-AKI [9].

In our study we compared two groups, who were using ACEI and CCB as monotherapy; and found no significant difference in development of CI-AKI. But this can be an effect of ACEI as well as CCB. ACEI also have been used to prevent CI-AKI in the past. Gupta et al. conducted a study of 71 patients who underwent coronary angiography and concluded that ACEI is effective against CI-AKI compared to placebo [19]. But more recent studies find no beneficial effect of ACEI. Furthermore Toprak et al. reported that in a randomized controlled study of 80 patients included; five patients (8.3%) in the ACEI group and 1 patient (3%) in control group developed CI-AKI and this difference was statistically significant ($p=0.02$). They concluded that using ACEI is a risk factor for development of CI-AKI [20]. In our study, 8 (15.7%) patients in CCB group and 7 (24.1%) patients in ACEI group developed CI-AKI. The two groups were similar in terms of CI-AKI development rates statistically ($p = 0.383$). This may be the result of both drugs lowering the risk factor of CI-AKI equally.

Age is a direct risk factor of developing CI-AKI. Especially elderly patients older than 70-75 years are at risk of developing CI-AKI [7]. Hui et al. showed in their study that amlodipine, a CCB, may decrease the risk of developing CMN in elderly patients [21]. In our study, the mean age of the CCB group was significantly higher than the ACEI group. The mean age of the patients in CCB group was 62.6 ± 12.6 , against the mean age of the patients in ACEI group was 56.5 ± 11.5 ($p = 0.01$). This could mean that CCB reduced the risk of older patients in the CCB group to a younger age risk level. Therefore two groups statistically appear indifferent in CI-AKI development rates.

Oguzhan et al. compared hydration therapy alone, versus valsartan-amlodipine combination plus hydration treatment in patients who have stage-II chronic kidney disease and going through coronary angiography. CI-AKI rates was 17.8% (n=8) in the CCB/ARB plus hydration group and 6.7% (n=3) in the only hydration group. As a result, they showed that amlodipine and valsartan treatment did not decrease the risk of CI-AKI [22]. Davidson et al. prospectively examined 1144 patients undergoing cardiac catheterization. They showed that the risk of developing CI-AKI does not decrease in patients using various CCB drugs [23]. In their prospective randomized study, Arıcı et al. [18] divided the 29 patients into two groups of amlodipine (n=15) and placebo (n= 14). Only one patient developed CI-AKI in each group. Two groups were similar statistically regarding CI-AKI development rates (amlodipine group: 6.6% n=1; placebo group: 7.1% n=1). Although our study was designed retrospectively, it is important to show similar results and rates with these three prospective studies.

The main limitations of our study are retrospective study design and relatively small sample size. Also; age difference between two groups and the fact that we could not randomize groups for contrast dose and their hydration status is a limitation of our study.

Conclusion

In our study, we evaluated one of today's important dilemma; the methods related to the prophylaxis of CI-AKI. Our study shows that there is no difference in the development of CI-AKI between patients using the CCB and ACEI as monotherapy. However, in our study, the mean age of patients using CCB was significantly higher than the group using ACEI. The volume expansion method is the most

favorable method for reducing the risk of CI-AKI and the pathophysiology behind CI-AKI is not fully explained. Therefore we need more studies exploring CI-AKI prophylaxis.

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ORCID iD of the author(s)

Burçin MA Atak Tel / 0000-0003-4201-9757

Memnune Sena Ulu / 0000-0003-0085-2193

Sinan Kazan / 0000-0001-7290-4680

Mehmet Polat / 0000-0003-1648-5543

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Investigation of therapeutic effect of *Saccharomyces boulardii* and translocation in immunosuppressed rats infected with *Shigella sonnei*

Mustafa Behcet¹  · Ayse Demet Kaya²

¹Department of Medical Microbiology, Bolu Abant Izzet Baysal University, School of Medicine, Bolu, Turkey

²Department of Medical Microbiology, Okan University, School of Medicine, Istanbul, Turkey

ABSTRACT

Aim: To investigate the therapeutic effects of *Saccharomyces boulardii* (*S. boulardii*) and detect blood and tissue penetrations of *S. boulardii* and *Shigella sonnei* (*S. sonnei*) in immunosuppressed rats infected with *S. sonnei*.

Methods: Forty rats were divided into four groups: Group A (immunosuppressed, not-treated); Group B (immunosuppressed, treated with *S. boulardii*); Group C (immunosuppressed, infected with *S. sonnei*, treated with *S. boulardii*); Group D (immunosuppressed, infected with *S. sonnei*). After taking samples for blood cultures, the rats were sacrificed. The large bowel, liver, spleen and mesenteric lymph nodes (MLN) were removed for microbiological examination.

Results: *S. boulardii* in group B and *S. sonnei* in group D were isolated from blood in some rats. Statistical analysis of our data, showed that the numbers of translocated colonies in the liver and spleen were relatively higher for *S. boulardii* in Group B and for *S. sonnei* in Group D, without reaching levels of statistical significance. For MLN, colony counts in Group B was higher than Group C and A showing statistical significance.

Conclusion: The administration of *S. boulardii* showed promising results for the therapy of *S. sonnei* infection in immunosuppressed rats, but therapeutic usage of *S. boulardii* should be carefully assessed by taking into consideration the risk it poses versus potential benefits in risk groups.

Keywords: *Saccharomyces boulardii*, *Shigella sonnei*, immunosuppression, rat, translocation.

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✉ Dr. Mustafa Behçet

Depart. of Medical Microbiology, Bolu Abant Izzet Baysal University, School of Medicine, Bolu, Turkey

E mail: mustafabehcet@ibu.edu.tr

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Introduction

Intestinal infections due to *Shigella spp.* are worldwide endemic but it mainly occurs in developing countries. Four species of *Shigella*

(*S.*; *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*) are the causative agents of shigellosis. Nearly two-thirds of the infections are caused by *S. flexneri* in low and middle-income countries.

S. sonnei is the leading species in high-income countries and the second most common species in low and middle-income countries. Immunodeficiencies lead to more severe clinical manifestations of *Shigella* infection

including persistent or recurrent intestinal disease and bacteriemia [1]. Antimicrobial agents used as effective options in the treatment of shigellosis became limited due to global drug resistance [2].

Saccharomyces boulardii (*S. boulardii*) is a non-pathogenic yeast used in many countries in the treatment of non-specific diarrhea and in cases of gut flora impairment. Several mechanisms such as fungal antagonism, diminution of the pathogenic effects of bacterial toxins, stimulation of intestinal immune defenses and increased intestinal disaccharidase activity can possibly explain the actions of *S. boulardii* in diarrhea. The activity of *S. boulardii* has been extensively studied in the contexts of gastroenteritis and antibiotic-associated diarrhea. The good tolerability of this yeast was shown and no serious adverse reactions have been reported despite a very slight potential risk of blood penetration of *S. boulardii* in immunodeficient individuals [3]. This study was conducted with the aim of investigating the therapeutic effects of *S. boulardii* in immunosuppressed rats infected with *S. sonnei* and detecting the presence of translocations of *S. boulardii* and *S. sonnei* in vulnerable hosts.

Materials and methods

These experiments were performed with the approval of the Ethics Committee of Duzce University School of Medicine (Decision no: 100-019). The animals which were used in the study were provided by Duzce University. The procedures were conducted according to routine animal care guidelines, and all experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996).

In this study, forty male Wistar albino rats weighing 200 ± 20 g were divided into four

groups of 10 animals each: Group A (immunosuppressed, not treated), Group B (immunosuppressed, treated with *S. boulardii*), Group C (immunosuppressed, infected with *S. sonnei*, treated with *S. boulardii*) and Group D (immunosuppressed, infected with *S. sonnei*).

All rats were housed individually in stainless steel cages in an animal room at 20^o C with a 12-hour light-dark cycle. All the rats were fed with a laboratory pellet diet and were allowed to have free access to water during the course of the experiments. Rats were decontaminated from antibiotics for 4 days by adding 2 mg/ml of streptomycin sulphate (Sigma, St. Louis, MO, USA) and 1500 units/ml penicillin-G (Sigma) to their water, which was prepared daily. Reduction of the gastrointestinal flora was confirmed by microscopic examination of Gram stained smears of fecal pellets and with aerobic cultures of feces for Gram-negative enteric bacteria.

The animals in all groups were given cyclophosphamide (Baxter Oncology, Germany) intraperitoneally at a dose of 200mg/kg. Group A was the immunosuppressed rats with no infection. On the fourth day after immunosuppression, Groups C and D were inoculated with 0.1 ml of *S. sonnei* containing 9×10^8 viable cells by gavage route after having cultures of the bacteria in Brain Heart Infusion broth (HiMedia Laboratories, India) for 12 hours [4]. On the third day after the inoculation, *S. sonnei* was isolated from the fecal samples of all rats.

Lyophilized *S. boulardii* (Ultra-Levure; BIOCDEX Laboratories, Montrouge, France) was given to each rat at a single dose of 10mg/day by gavage route for Groups B and C. Group A was given 0.1 ml (same amount as other groups) of phosphate buffer saline (PBS), as this group was neither inoculated by *S. boulardii* nor by *S. sonnei*. *S. boulardii*

administration was continued for 10 days for group B and for five days for group C. The rats were sacrificed under ether inhalation on day 10.

Prior to being sacrificed, blood samples (2 ml) of rats were collected from inferior vena cava and inoculated into the bottles of BACTEC system (Becton Dickinson, Ireland).

After the rats were sacrificed; their large bowels, liver, spleen and MLN were removed for microbiologic examination. To evaluate the translocation by microbiological methods; the weights of the liver, spleen and nodes were recorded.

To assess *S. boulardii* and/or *S. sonnei* quantitatively, tissue pieces were minced with a scalpel, diluted by tenfold in 0.9% NaCl and homogenized with a handled tissue tearer. (Ultra-Turrax T25, BioSpec Products, Bartlesville, OK, USA).

Diluted organ homogenates were transferred on Sabouraud dextrose agar (SDA) (HiMedia Laboratories, India) and Hektoen Enteric (HE) (HiMedia Laboratories, India) agar plates. The duration of the cultures were as follows: 7 days for blood cultures, 72 hours for SDA and 48 hours for HE agars at 35°C. Microorganisms were identified by conventional methods and API 32E (BioMerieux, France) and API CAUX (BioMerieux, France) systems. Then, the number of colonies per tissue gram (cfu / g) was determined.

The translocation index shows the number of microorganisms per gram of tissue and was calculated by the following formula [5]:

$$\text{(Cfu count} \times \text{dilution coefficient} \times 10 \times 2) / \text{Tissue weight.}$$

Statistical Analysis

The data was expressed as means \pm SD. Kruskal-Wallis and Mann-Whitney U tests were used to compare the numbers of colonies

between the groups. The differences were considered as being significant at $p < 0.05$.

Results

The number of colonies of the microorganisms isolated from the cultures of MLN, liver and spleen specimens and the results of blood cultures were determined for each group.

In Group A, in immunosuppressed rats, blood and tissue cultures were negative and no translocations were seen.

In Group B (immunosuppressed, treated-with-*S. boulardii*), the results showed that *S. boulardii* translocated and it systemically spread to extraintestinal sites of liver, spleen and MLN in some rats. Of 10 rats, one yielded positive results in the liver, spleen, MLN and blood; one yielded positive results in spleen and MLN; one yielded positive results in MLN and blood; and, lastly, four rats yielded positive results in only the MLN cultures. The isolated organism was *S. boulardii* from the MLN, liver and spleen specimens. *S. boulardii* was also isolated from the blood specimens of two rats in which translocations were observed.

In Group C (immunosuppressed, infected-with-*S. sonnei*, treated-with-*S. boulardii*), neither *S. boulardii* nor *S. sonnei* was isolated from the cultures of MLN, liver, spleen and/or blood specimens.

In Group D (immunosuppressed, infected-with-*S. sonnei*), the results of the cultures were as follows: one rat yielded positive results in the liver, spleen, MLN and blood specimens; one yielded positive results in the liver, MLN and blood specimens; and one rat yielded positive results in MLN for *S. sonnei*.

The numbers of translocated colonies are listed in Table 1 and are expressed as cfu. The numbers of translocated colonies in liver and spleen were relatively higher for *S. boulardii* in Group B and higher for *S. sonnei* in Group D,

but the difference was not statistically significant ($p > 0.05$). For MLN, colony counts in Group B was higher than in Groups C and A, which was statistically significant ($p = 0.005$). In Group D, colonies of *S. sonnei* was high, but not with statistical significance ($p = 0.068$).

certain agents, including *Giardia intestinalis*, Rotavirus, *Entamoeba histolytica*, *Helicobacter pylori*, *ETEC*, *Cryptosporidium parvum* as well as in the treatment of diarrhea associated with HIV [9-11]. *S. boulardii* has been found to show a protective effect in *Clostridium difficile*

Table 1. Numbers of colonies in the liver, spleen, MLN and blood in the four experimental.

Parameters	A (n=10)	B (n=10)	C (n=10)	D (n=10)
Liver* (cfu/g/10 ⁵)	0.00	363.60±1149.80	0.00	194.20±409.41
Spleen ** (cfu/g/10 ⁵)	0.00	1039.10±2360.01	0.00	210.50±666.65
MLN*** (cfu/g/10 ⁵)	0.00	47273.70±75043.94	0.00	10378.70±17400.4
Bacteriemia/fungemia	0	2	0	2

n= number of rats. **B**: For *S. boulardii*

*** ($p = 0.001$) (Kruskal-Wallis test); ($p = 0.005$) groups A-B, B-C (Mann-Whitney U test)

D: For *S. sonnei*

*** ($p < 0.05$) (Kruskal-Wallis test): ($p = 0.068$) groups A-D, C-D (Mann-Whitney U test)

Discussion

S. boulardii is widely used for clinical conditions and predominantly for the prevention of diarrhea [6,7]. The effects of *S. boulardii* are related to its inhibitory effects on the growth of intestinal microorganisms and to the neutralization of toxins, however the mechanisms underlying such effects could not be fully identified. Furthermore, *S. boulardii* has been shown to increase the activity of brush border disaccharidases in human volunteers and in patients with congenital sucrase isomaltase deficiency. In addition, an enhanced release of secretory IgA into the intestinal lumen supports the immune system [8].

Many studies have been conducted to investigate the activity of *S. boulardii* against

associated colitis [12,13]. Studies showed a protective effect against *Salmonella typhimurium* and *Shigella flexneri* in the intestinal tracts of conventional or gnotobiotic mice [14]. The effects of orogastric administration of *S. boulardii* on the jejunal villi was studied by Dias et al. [15] in rats infected with *Vibrio cholerae* and their data showed the inhibition of the action of the cholera toxin on enterocytes by *S. boulardii*. Sheele et al. [16] showed the efficiency of *S. boulardii* in patients in whom the duration and severity of cholera was reduced. Zbinden et al. [17] investigated the influence of *S. boulardii* on *Salmonella typhimurium* and *Yersinia enterocolitica* under in vitro conditions and

their results showed that *S. boulardii* inhibited either the growth of both bacteria or their invasion into HeLa cells, so they suggested to study these effects in vivo as well.

In recent years, invasive fungal infections have been frequently reported worldwide in parallel to with the increase in risky population such as patients with chronic or debilitating diseases, who receive immunosuppressive drugs broad spectrum antibiotics, and parenteral nutrition and who were administered central venous catheter [18].

Microbial translocation is defined as the passage of viable microbes from the gastrointestinal (GI) tract to extraintestinal sites, such as the MLN, spleen, liver, kidneys, and blood [19]. Overall, *S. boulardii* is considered to be a safe and well tolerated agent, but recently, numerous studies reported fungemia after *S. boulardii* treatment for risk groups [17,20-28]. Some studies emphasize the importance of blood penetration after *S. boulardii* usage in immunosuppressed patients resulting in *S. boulardii* associated fungemia [29,30]. For this reason, the therapeutic usage of probiotics should be carefully evaluated by taking into consideration its risks as well as its potential benefits.

In a study investigating the ability of orally administered viable *S. boulardii* in inhibiting translocation of *Candida albicans* from the gastrointestinal tract in antibiotic-decontaminated, specific pathogen-free mice, orally administered *S. boulardii* was shown to decrease the incidence of *Candida albicans* translocation to the MLN, liver and kidneys [19].

Peret Filho et al. [31] studied the translocation and histological alterations in the terminal ileum, liver and spleens of immunosuppressed mice under *S. boulardii* treatment. The results of this study showed that *S. boulardii*

administration decreased the bacterial translocation to the liver and spleen in a dose dependant manner. Low *S. boulardii* translocation to MLN was observed in some animals. In our study, in Group B, translocation to MLN was significantly higher ($p=0.005$). The translocation to spleen and liver was also high, but the difference was not statistically significant ($p>0.05$). In evaluation of the blood cultures, two rats (20%) in Group B developed fungemia due to *Saccharomyces cerevisiae* representing the translocation of *S. boulardii*. Comparing our results with Peret et al. [31] our findings also support the relative protection with *S. boulardii* in immunosuppressed rats. However, reported cases in clinical trials and development of fungemia in two immunosuppressed rats showed the importance of probable fungemia during treatment.

Shigellosis is currently an important public health problem. Shigella bacteriemia is rare but associated with a high mortality rate. Immunosuppressed patients, malnourished children, and elderly people are at risk of serious complications and bacteriemia [2,32]. In experiments on white mice, *S. sonnei* strains were shown to be capable of penetrating into the blood for a short period of time [33]. Over the past decades, Shigella strains have progressively become resistant to most of the widely used antimicrobials [2]. For this reason, alternative therapeutic approaches are under investigation. In our study, we investigated the therapeutic effects of *S. boulardii* in *S. sonnei* infection, which is the predominant cause of Shigellosis in our country and the translocation rate of *S. sonnei* and *S. boulardii* in immunosuppressed hosts. The results of our study showed that in some rats in the D group *S. sonnei* cause bacteremia and caused translocation in the MLN, liver and spleen. These results showed the importance of

bacterial translocation in immunosuppressed rats. In group C, in which the immunosuppressed rats were infected with *S. sonnei* and treated with *S. boulardii*, no translocation and no growth in blood cultures was observed.

Conclusion

Both *S. boulardii* and *S. sonnei* caused translocation resulting in fungemia/bacteriemia in immunosuppressed rats when they were applied individually; but *S. boulardii* administration to *S. sonnei* infected rats seemed to result in the inhibition of translocation and bacteriemia. *S. boulardii* administration is found to be effective in the treatment of *S. sonnei* infections in immunosuppressed rats under in vivo conditions, but potential risk of developing fungemia in risk groups must always be considered during the therapeutic use of *S. boulardii*.

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ORCID iD of the author(s)

Mustafa Behcet / 0000-0002-5676-6983

Ayşe Demet Kaya / 0000-0001-8224-8242

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Investigation of the effect of REM sleep deprivation on epileptic seizures caused by pentylentetrazole in mice

Recep Akkaya¹  · Sebahattin Karabulut² · Ahmet Sevki Taskiran³

¹Department of Biophysics, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey

²Department of Medical Services and Techniques, Sivas Cumhuriyet University, Vocational School of Health Services, Sivas, Turkey

³Department of Medical Physiology, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey

ABSTRACT

Aim: To investigate whether different periods of rapid eye movement sleep deprivation (REM SD) contribute to seizure susceptibility, hippocampal oxidative status and balance of inhibition-excitation in the acute epilepsy model.

Methods: REM SD was performed using the modified multiple platforms method on adult male BALB/c mice. Pentylentetrazol (PTZ) was injected to induce seizures and hippocampal total antioxidant status (TAS), total oxidant status (TOS), gamma aminobutyric acid (GABA), and glutamate levels were measured using the ELISA method.

Results: PTZ-induced seizures following 8 h and 72 h REM SD significantly reduced the hippocampal TAS levels, but did not affect the TOS levels. In REM SD groups, especially after 8 hours of REM sleep loss, there was a significant increase in glutamate in PTZ induction. The hippocampal GABA levels were increased by PTZ-induced seizures after 72 h REM SD. PTZ-induction after 8 hours of RAM SD leads to a significant increase in the seizure duration.

Conclusion: It can be speculated that the REM SD can contribute to seizure susceptibility by changing the oxidant-antioxidant balance and excitatory and inhibitory tone in the hippocampus.

Keywords: REM sleep deprivation, hippocampus, seizure susceptibility, oxidative stress.

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✉ Dr. Recep Akkaya

Department of Biophysics, Sivas Cumhuriyet University,
School of Medicine, Sivas, Turkey

E mail: recepakkaya5835@gmail.com

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Introduction

Epilepsy is one of the most common neurological disorders characterized by recurring seizures that originated due to

imbalances in excitation and inhibition in the brain [1,2]. Despite the presence of existing antiepileptic drugs (AEDs) that can successfully prevent recurrent seizures in the majority of patients, about a third of patients are resistant to treatment [3]. Also, AEDs do not affect the underlying pathophysiology and progression of the disease, they often provide symptomatic therapy [4]. Current studies aim to elucidate the cellular mechanisms in which a

normal brain is epileptic, that is, epileptogenesis. This term refers to a process that progressively alters neuronal excitability. The development of antiepileptogenic drugs that prevent or reduce the progression of the disease could have a major effect on the life of patients with epilepsy. Therefore, understanding the cellular mechanisms of epileptogenesis and its treatment are research priorities on the political agendas in both Europe and the United States [5].

Sleep loss is widespread in neurodegenerative diseases and pieces of evidence indicate that sleep interacts with disease and is not only a symptom of it [6]. It has been reported that sleep deprivation (SD) can alter cortical excitability, which is seen as the balance between the inhibitor and excitatory of neuronal circuits in the cortex, which can reduce the epileptic threshold [7]. Interestingly, the seizures are less common in rapid eye movement (REM) sleep, so a seizure-protecting role has been proposed for this sleep phase [8]. Although SD is thought to promote neurodegeneration, not much is known about how they interact mutually. Therefore, in this study, we aimed to investigate whether different periods of REM SD contribute to seizure susceptibility. For this purpose, we evaluated epileptic behaviors induced by pentylentetrazol (PTZ) and examined the oxidative status and inhibition-excitation balance in the hippocampus.

Materials and Methods

The study was performed with 24 adult male, weighing 35-38 g, BALB-c Albino mice (obtained from Cumhuriyet University Animal Laboratory). Five mice per cage were housed under controlled environmental conditions with regard to temperature ($23 \pm 2^\circ\text{C}$), humidity (35%-60%), and a 12:12 h light–dark cycle.

The mice were allowed access to food water and libitum. All procedures were performed in accordance with the guidelines of the Local Ethics Committee for the welfare of experimental animals (Registry Number: 65202830-050.04.04-166 dated 09.04.2018). All efforts were made to decrease the number of animals used to a minimum. The procedures were conducted according to routine animal care guidelines, and all experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996).

Experimental procedure

The mice were randomly assigned to into four groups each containing six animals as follow: The control group, not deprived of REM sleep; the PTZ group, PTZ injected; the REM SD 8+PTZ group, PTZ injected after deprived of REM sleep for 8 h; the REM SD 72+PTZ group, PTZ injected after deprived of REM sleep for 72 h.

REM SD procedure

The modified multiple platform method was used to restrain REM sleep for 8 h and 72 h [9]. The animals were placed in a modified multi-platform box made of plastic (50 cm in length and 30 cm in height). Ten cylindrical small platforms (3 cm in diameter and 10 cm in length) were positioned on the tank floor. As the platforms will remain 2 cm above the surface, the container was covered with water (22°C) 8 cm in depth. Standard laboratory food blocks and a bottle of water were provided to animals to access food and water ad-libitum. The mice were placed on small platforms where they can move. When REM sleep began and after losing muscle tone, the mouse on the platform came into contact with water and awakening. Previous experiments have shown that the large platform used as the standard control reduces

approximately 80% of the REM sleep [10]. Therefore, the control mice were housed in their cages separated in the experimental room to be provided in the same environment but benefited their normal sleep period.

Induction of seizures

To induce seizures, PTZ was injected intraperitoneally (i.p.) at a convulsive dose of 60 mg / kg. After each injection, the mice were individually placed in plexiglass cages for 30 minutes of observation, then the first myoclonic jerk (FMJ), generalized tonic-clonic seizure latencies (GTCS1) and GTCS (GTCSd) duration were recorded. The development of seizure and status epilepticus was evaluated by a behavioral (Racine's Convulsion Scale (RCS)) as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks; 4 = tonic-clonic seizure while the animal remained on its feed; 5 = tonic-clonic seizure with loss of the righting reflex; and 6 = lethal seizure. At the end of the experiment, the animals were sacrificed using the decapitation method and their brains were isolated for further evaluation of biochemical parameters.

Biochemical Analysis

Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) were used as markers for demonstrating the antioxidant and oxidative capacities. The percent ratio of TOS to TAS was accepted as the Oxidative Stress Index (OSI), a marker of the severity of oxidative stress. OSI was calculated as follows: $OSI = [(TOS, \mu\text{mol H}_2\text{O}_2 \text{ Eq/mg protein}) / (TAS, \mu\text{mol Trolox Eq/mg protein}) \times 100]$ and evaluated as an indicator of the degree of oxidative stress [11]. Brain tissue samples from each group were homogenized within 10

volumes of the ice-cold homogenization buffer and centrifuged at $12,000 \times g$ for 10 min at 4°C . The supernatant was collected for protein concentration determination by a Bradford protein assay kit (Merck, Germany). Hippocampal antioxidant and oxidant status was determined by using TAS and TOS Assay Kits (Rel Assay Diagnostics® Mega Tip Ltd., Gaziantep, Turkey). Hippocampal GABA and Glutamate levels were analyzed by using gamma aminobutyric acid (GABA) and Glutamate Assay Kits (Rel Assay Diagnostics® Mega Tip Ltd., Gaziantep, Turkey).

Statistical analysis

The data were presented as the standard deviation of the mean (SEM). The RCS score, FMJ time, duration of GTCS, GTCS latency, and the levels of hippocampal protein were evaluated using a one-way analysis of variance (ANOVA). All statistical analyses were performed using IBM SPSS Statistical software Version 22.0 (IBM, Armonk, NY, USA). A p-value of less than 5% was accepted statistically significant.

Results

Behavioral measurements

The effect of REM SD on PTZ-induced seizure parameters is shown in Figure 1 A-D). There was no significant difference between the four groups in the RCS, FMJ, and GTCS1 results, but there was a significant increase in the GTCSd results between the REM SD 8 + PTZ group compared to the PTZ group ($p < 0.05$).

Hippocampal TAS, TOS, GABA, and glutamate expressions

The oxidant stress parameters obtained from the hippocampus are given in Figure 2A-C. The TAS levels of REM SD 8 + PTZ and REM SD 72 + PTZ groups decreased significantly in the hippocampus compared to the control

group ($p < 0.001$ and $p < 0.05$, respectively). There was a significant decrease in the hippocampal antioxidant capacity in the REM SD 8 + PTZ group compared to the PTZ group ($p < 0.05$). There was no significant difference in the hippocampal TOS levels between the four groups.

There was a significant increase in OSI levels of the REM SD 8 + PTZ group compared to the control and the PTZ groups ($p < 0.01$).

A significant increase in the expression of GABA was found in the REM SD 72+PTZ group ($p < 0.05$).

The increase in the hippocampal glutamate level in the REM SD 8 + PTZ group was statistically significant ($p < 0.001$). There was a significant increase in the hippocampal glutamate level in the REM SD 72+PTZ group compared to the control group ($p < 0.01$) (Figure 3A,B).

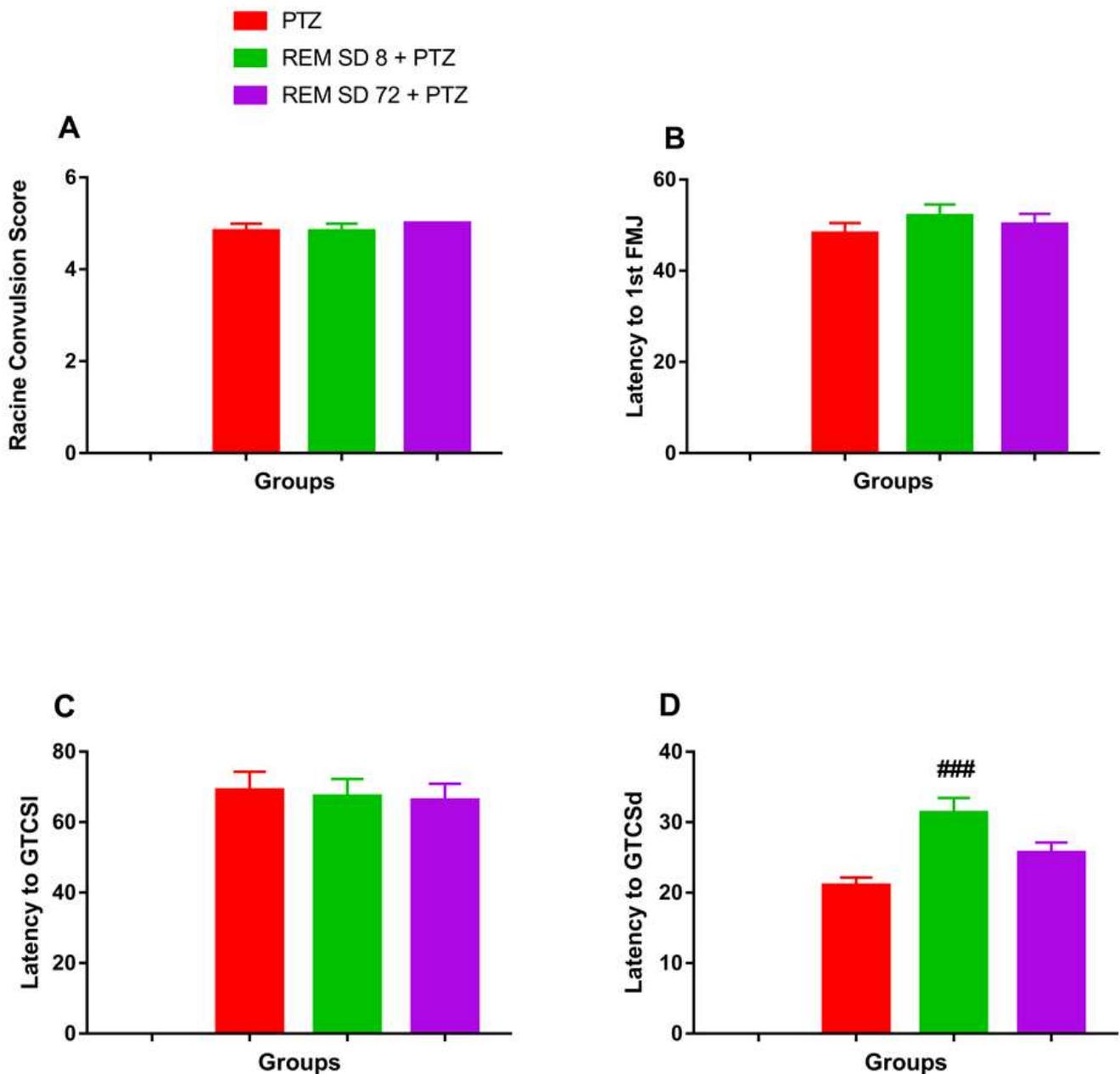


Figure 1A-D. Effects of REM SD on seizures threshold (latency) and duration in PTZ-induced seizures in mice. Data expressed as mean \pm SEM. n=6. ### $p < 0.001$ compared with PTZ group.

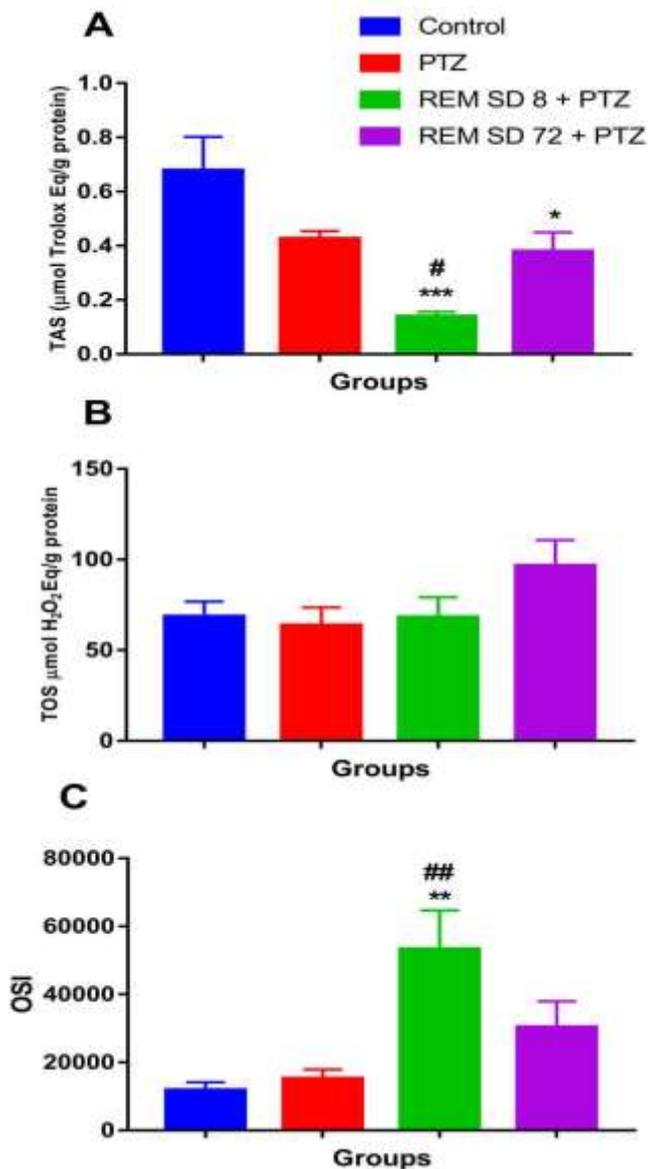


Figure 2A-C. Effects of REM SD and PTZ on hippocampal TAS, TOS, and OSI levels. Data expressed as mean ± SEM. n=6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with Control; # $p < 0.05$, ## $p < 0.01$ compared with and PTZ group.

Discussion

In the present study, we explored the effect of REM SD on seizure susceptibility in the acute epilepsy model. Further, we investigated the effects of PTZ-induced seizures following 8 h and 72 h REM SD on excitatory-inhibitory and oxidant-antioxidant balance in the hippocampus. We found that seizure induction after relatively short (8 h) and long (72 h) period REM SD differently affect the oxidant

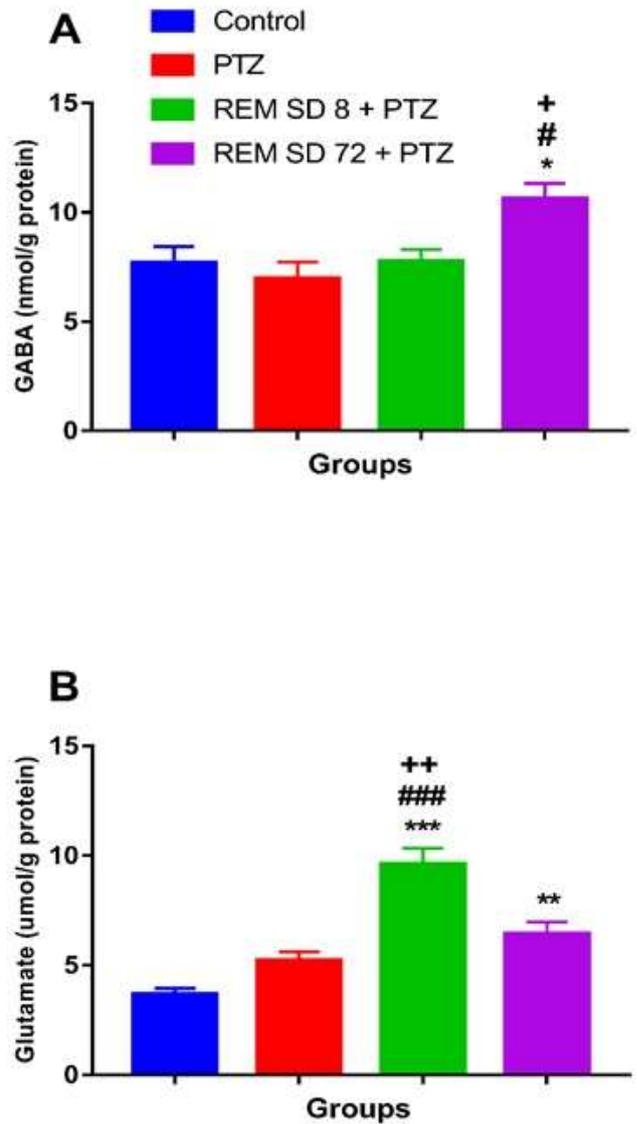


Figure 3A-B. Effects of REM SD and PTZ on hippocampal GABA and Glutamate expressions. Data expressed as mean ± SEM. n=6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with Control group; # $p < 0.05$, ### $p < 0.001$ compared with PTZ group; + $p < 0.05$, ++ $p < 0.01$ compared with REM SD 72+PTZ group.

and antioxidant capacities in the hippocampus. This result was also similar to in hippocampal excitatory and inhibitory tone. The findings of the present study also illustrated the time of REM SD may affect seizure susceptibility. Oxidative stress is a highly disordered metabolic process characterized by increased cellular reactive oxygen species [12]. This event eventually generates an imbalance

between oxidants and antioxidants. It is well established that both sleep loss and PTZ-induced seizure increase oxidative stress in the brain [13,14]. We detected only minimal differences in hippocampal TOS levels in this study that did not reach statistical significance. However, our results showed that PTZ-induced seizures after 8 h and 72 h REM SD led to a significant decrease in hippocampal TAS levels. It has been assumed that oxygen free radicals accumulate during awake as a result of utilized great amounts of oxygen, and sleep allows the removal of these reactive oxygen species in the brain [13]. Therefore, the wakefulness result from sleep loss disrupts the oxidant and antioxidant balance in the brain. Ramanathan et al. showed that long term (5-11 days) total SD decreased an antioxidative enzyme superoxide dismutase (SOD) activity in the rat hippocampus [15]. In agreement with our results, Suer et al. reported that 21-day REM SD leads to a decrease in the antioxidant defenses in the hippocampus [16]. Moreover, an unexpected but intriguing finding was that the effect of PTZ-induced seizures after 8 h REM SD was more prominent than 72 h REM SD on the hippocampal TAS and OSI levels. This unexpected finding can be explained by that the long-term SD may lead to an adaptive reaction of the brain against sleep loss. The present hippocampal GABA results support this hypothesis as its level was increased by PTZ administered following 72 h REM SD. The PTZ-induced seizures after 8 h REM SD also caused a considerable increase in the hippocampal glutamate (an excitatory transmitter) levels. In line with our results, 6- and 12-h total SD resulted in a significant increase in the hippocampal glutamate levels [17]. Taking account that PTZ-induced seizures after the 72 h REM SD also lead to an increase GABA level in the hippocampus, we think that

REM SD for 8 h has a more potent effect than REM SD for 72 h on seizure susceptibility.

In the present study, we also found that the seizures durations were significantly increased after the 8 h REM sleep loss. The finding implies that REM sleep a critical sleep period for an epileptic phenomenon. In agreement with our results, Marcus et al. concluded that seizures are less common in REM sleep, and this stage of sleep plays a protective role against generalized seizures [8]. However, we could not find a correlation between the REM SD duration and biochemical and behavioral results tested herein. The plausible reason for this could be attributed to the fact that the longer period of REM SD induces a compensatory response that provides resistance to the occurrence of epileptic seizures. It should be noted that the modified multiple platform procedure used in the present study can interfere with the majority of REM sleep, besides it can restrain up to 40% of NREM sleep [10]. Thus, the NREM sleep deprivation may have also contributed to our results.

Conclusion

In conclusion, we report that the REM SD may affect seizure susceptibility by altering the balance of the hippocampal oxidant-antioxidant, and stimulant and inhibitor. In addition to the acute epilepsy model, investigating seizure susceptibility in a chronic epilepsy model may value further studies in rodent epileptogenesis and SD models.

Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *This experimental study was reviewed and approved by the local ethics committee (Registry Number: 65202830-050.04.04-166 dated 09.04.2018).*

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ORCID iD of the author(s)

Recep Akkaya / 0000-0002-3477-7198

Sebahattin Karabulut / 0000-0002-3261-4125

Ahmet Sevki Taskiran / 0000-0002-5810-8415

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Prognostic value of the optic nerve sheath thickness as an indicator of intracranial pressure among acute stroke patients

Kaan Celik · Tamer Colak ·  Beliz Oztok Tekten

Department of Emergency, Bolu Abant Izzet Baysal University, Medical Faculty, Bolu, Turkey

ABSTRACT

Aim: To evaluate the clinical value of optic nerve sheath thickness (ONST) in stroke patients.

Method: The present study was prospectively performed on 386 stroke patients who were admitted to Emergency Department, and 75 healthy volunteers of similar age and gender groups. The following criteria were evaluated for each patient: age, gender, comorbidities, neurological deficit levels [Glasgow Coma Scale (GCS), the National Institute of Health Sciences Scale (NIHSS)], tomography findings (hemorrhagic / ischemic), ONST diameter in ultrasound scan, hospitalization and mortality rates.

Results: The median ONST value was 5.5 mm (IQR: 0.30) for the hemorrhagic stroke patients, and 5.25 mm (IQR: 0.20) for the ischemic stroke patients. The median ONST value of the stroke patients was significantly higher. Also, the ONST values of the hemorrhagic stroke patients were found to be significantly higher. In our study, the area below the curve was 0.865. For the 4.5 mm cut-off value, the sensitivity was 96.1% and specificity was 82.7%.

Conclusions: The results of our study showed that the ONST increased in the stroke patients and this increase was higher in the hemorrhagic stroke patients. We suggest that the treatment can be considered to decrease intracranial pressure if there is an increase in ONST in stroke patients.

Keywords: Stroke, optic nerve sheath thickness, ultrasound, emergency medicine.

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✉ Dr. Tamer Colak

Department of Emergency, Bolu Abant Izzet Baysal University, Medical Faculty, Bolu, Turkey

E mail: drtamercolak@gmail.com

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Introduction

Stroke is the third most common cause of mortality, and the most common cause of morbidity worldwide [1]. Among all stroke patients, 80-87% are ischemic, whereas remaining patients are hemorrhagic [2,3].

Intracranial pressure (ICP) arises from pressure of the cerebrospinal fluid, blood and brain tissue. ICP can increase due to cranial trauma, stroke, the presence of a mass and infection; due to both primary reasons and the released mediators. This increase in ICP increases morbidity and mortality [4].

Emergency services commonly use imaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI) for the diagnosis of stroke and the determination of ICP; however, the use of these methods is very

limited due to the potentially unstable condition of the patients, the low sensitivity and specificity of these methods regarding ICP in many patients, and the low reproducibility [5]. ICP needs to be quickly determined and lowered as it can increase mortality. The most reliable measurement of ICP is through invasive procedures; however, the associated complications (including coagulopathy and local infections) and the requirement of special devices make it inefficient to use this method in the ER setting [6]. Therefore, researchers are constantly trying to develop new methods for the determination of ICP.

The optic nerve is part of the central nervous system; it is covered with dura mater and encompassed with the subarachnoid CSF [6]. It has been reported that increasing ICP increases the thickness of the optic nerve sheath (ONST), and that this correlation is significant [7-9]. This expansion can be measured specifically clearly at the retrobulbar level [10]. Therefore, in this prospective study, we aimed to investigate the potential value of ONST in the approach to diagnosis and treatment as an indicator of increased ICP in stroke patients admitted to our emergency clinic.

Materials and methods

The present study was prospectively performed on 386 stroke patients who were admitted to Emergency Department of a public hospital in Turkey between 01.02.2018 and 30.05.2018, and 75 healthy volunteers of similar age and gender groups. This study was approved by the Ethical Committee of Bolu Izzet Baysal University Faculty of Medicine (Date: 08/02/2018; Decision number: 2018/12). The rights of all participants were protected and written informed consents were obtained before the study according to the Helsinki Declaration. The following criteria were evaluated for each

patient: age, gender, comorbidities, neurological deficit levels [Glasgow Coma Scale (GCS), the National Institute of Health Sciences Scale (NIHSS)], tomography findings (hemorrhagic/ischemic), duration to ultrasound scan [the period between referral and ultrasound (US) scan], ONST diameter in US, hospitalization and mortality rates. NIHSS and GCS score were used to determine neurological deficit levels. The main reason why US screening is preferred in this study is the ease of application at the bedside in the emergency room. In addition, absence of radiation and repeatability may provide use of patient follow-up. Moreover, value of US scan was tried to be supported by comparing the OSNDs measured by US scan and brain tomography.

The exclusion criteria were as follows: patients with transient ischemic attack (TIA), patients aged under 18, vascular dementia, patients with liver failure or chronic renal insufficiency, hypoglycemia, pregnant and nursing women, patients with trauma, traumatic optic neuropathy, optic neuritis, optic nerve arachnoid cyst, and patients with orbital/cavernous sinus cysts.

The patients with conditions that may cause intracranial pressure increase including hydrocephalus, arachnoid cyst, hypertensive encephalopathy, pseudotumor cerebri, intracranial tumor or metastases, intracranial abscess, the patients with history of central nervous system infection, neurosurgery; the patients with benign intracranial hypertension, cranial trauma, pathology which causes jugular compression and those with findings of intoxication were excluded.

A trained emergency medicine specialist applied a thin layer of gel to both eyes of the patients in the supine position and performed the measurement with a 7.5 MHz linear probe. The optic nerve sheath diameter was measured

from 3 mm behind the posterior part of the globe from the sagittal and transverse planes at the same point. The left and right optic nerve sheath diameters were calculated by using the median value of the transverse and sagittal measurements. This calculation yielded the median optic nerve sheath diameter. All images were controlled and confirmed by a blinded emergency medicine specialist, subsequently, the patient and control groups were compared. The widest ONSD appeared due to intracranial pressure increase was at 3 mm behind the globes; no significant dilatation was shown posterior than 3 mm [10]. Therefore, ONSD was measured at axial plane at 3 mm posterior to the optic nerve on both eyes from brain parenchyma frame; and average of the measurements was obtained.

The data were evaluated through SPSS (Statistical Package for Social Sciences) Windows 22.0 program. Distribution of the continuous variables was tested by Kolmogorov Smirnov test. The descriptive data were expressed in number of cases (n) and percentages (%). The quantitative data were presented in median and interquartile range (IQR). The continuous data were compared using the Mann Whitney-U, and the qualitative data were compared using the chi-square test. The relationship between the categorical variables and ONST were evaluated using the Spearman correlation analysis. An ROC curve was prepared to demonstrate the effectiveness of ONST in indicating ICP. The results were evaluated in a confidence interval of 95% and a significance level of $p < 0.05$.

Results

The median age of the patients in our study was 71 (IQR: 19), and 51.3% of the subjects were males. The two groups were similar regarding age, gender, presence of additional diseases,

Table 1. Comparison of demographic characteristics of the patients and control group.

Parameters	CVA (n=386)	Control (n=75)	p
Age, Median (IQR)	71 (19)	71 (19)	0,713
Gender Male, n (%) Female, n (%)	198 (51.3) 188 (48.7)	39 (52.0) 36 (48.0)	0,911
OSNT, Median (IQR)	5.25 (0.25)	4.1 (0.25)	<0,001
CCT OSNT, Median (IQR)	5.60 (0.65)		
Time (min), Median (IQR)	260 (80)		
GCS, Median (IQR)	15 (0)	-	
NIHSS, Median (IQR)	12 (7)	-	
Concomitant disease, n (%)	292 (75.6)	49 (65.3)	0.063
HT, n (%)	235 (60.9)	46 (61.3)	0.941
DM, n (%)	118 (30.6)	13 (17.3)	0.020
CAD, n (%)	94 (24.4)	14 (18.7)	0.287
AF, n (%)	31 (8.0)	1 (1.3%)	0.037
Previous CVA, n (%)	27 (7.0)	3 (4.0%)	0.336
COPD/Asthma, n (%)	17 (4.4)	1 (1.3%)	0.209
CHF, n (%)	16 (4.1)	0	0.073
Other, n (%)	6 (1.6)	0	0.277
SBP, The Median (IQR)	165 (32)	124 (28)	<0.001
DBP, Median (IQR)	90 (20)	80 (20)	<0.001
Pulse, The Median (IQR)	82 (15)	69 (17)	<0.001
Hospitalized in, Clinic n (%) ICU	286 (74.1) 100 (25.9)		
Death, n (%)	24 (6.2)		
ONST	5.25 (0.25)	4.1 (0.25)	<0.001

n: number of patients, ONST: nerve sheath thickness, CCT: Cranial computed tomography GCS: Glasgow Coma Scale, NIHSS: National Institute Of Health Sciences Scale, HT: Hypertension, DM: Diabetes Mellitus, CAD: Coronary Artery Disease, AF: Atrial Fibrillation, CVA: Cerebrovascular Accident, COPD: Chronic Obstructive Pulmonary Disease, CHF: Congestive Heart Failure, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, ONST: Optic Nerve Sheath Thickness.

hypertension (HT), previous strokes, coronary artery disease (CAD), congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD)/asthma, and other comorbidity rates

Table 2. Stroke type and the findings.

Type	Findings	CVA (n=386)
Haemorrhagic, n (%) 34 (8.8)	Intraparenchymal hematoma	18 (4.7)
	Haemorrhage opened into the ventricle	8 (2.1)
	Non-traumatic subarachnoid haemorrhage	8 (2.1)
	Shift larger than 3 mm	11 (2.8)
Ischemic, n (%)**352 (91.2)	Infarction of the middle cerebral artery	190 (49.2)
	Infarction of the anterior cerebral artery	10 (2.6)
	Vertebrobasillary artery	102 (26.4)
	Lacunar infarction	86 (22.3)
	Undetected	8 (2.1)
	Shift larger than 3 mm	22 (5.7)

* Cerebral magnetic resonance imaging and tomography data were collected.

** Since one case has multiple lesions, number of total cases is higher than the patients with ischemic stroke.

($p > 0.05$) (Table 1). In our study, the median ONST diameter value of the stroke patients was 5.25 mm median (IQR: 0.25), where the median ONST diameter of the control group was 4.1 mm (IQR: 0.25). The median ONST value of the stroke patients was significantly higher ($p < 0.001$). Median ONST diameter value of the stroke patients detected by CT was 5.60 mm median (IQR: 0.65). The period between clinical development and USS scan was 260 minutes (IQR: 80). The prevalence of diabetes mellitus (DM) and atrial fibrillation (AF) was significantly higher among the stroke patients ($p < 0.05$). The patient's median Glasgow Coma Scale (GCS) score was 15 (IQR: 0) and the median NIHSS score was 12 (IQR: 6.3). The systolic blood pressure (SBP), diastolic blood

pressure (DBP) and pulse values were found to be higher for the study group compared to the control group ($p < 0.05$). 74.1% of the patients were hospitalized in the clinic and is 25.9% were hospitalized in the ICU, 6.2% of the patients died (Table 1). In the patient group, 91.2% of stroke patients were ischemic and 8.8% were hemorrhagic. The most common cause for ischemia was infarction of the middle cerebral artery; the most common cause for hemorrhage was parenchymal hematoma (Table 2).

The median ONST value was 5.5 mm (IQR: 0.30) for the hemorrhagic stroke patients, and 5.25 mm (IQR: 0.20) for the ischemic stroke patients. The ONST diameters of the hemorrhagic patients were found to be significantly higher ($p < 0.001$) (Figure 1).

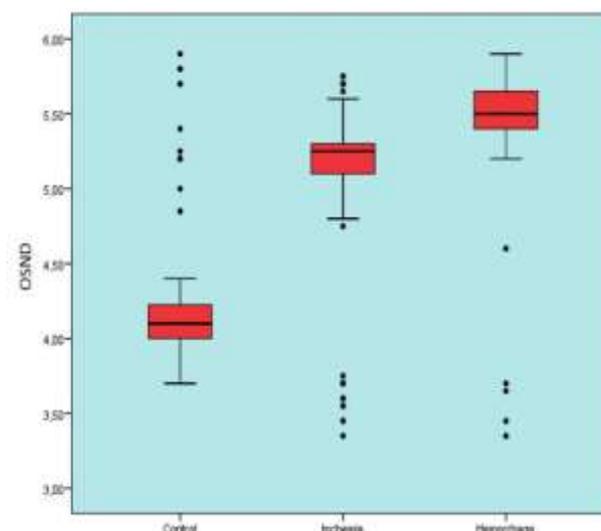


Figure 1. Comparison of the ONST groups.

In the present study (all groups including hemorrhagic and ischemic patients), there was no correlation between ONST diameter; age and pulse ($p > 0.05$). There was a negative correlation between ONST and GCS, and a positive correlation between ONST and ONST detected by CT, USS period, SBP, DBP, NIHSS ($p < 0.05$) (Table 3).

Table 3. The association between the ONST and demographic data.

Data	All subjects		Haemorrhagic		Ischemic	
	r	p	r	p	r	p
Age	0.003	0.947	0.555	0.001	-0.005	0.923
CCT ONST	0.704	<0.001	0.910	<0.001	0.702	<0.001
Time	0.168	0.001	0.777	<0.001	0.194	<0.001
GCS	-0.294	<0.001	-0.668	<0.001	-0.171	0.001
NIHSS	0.239	<0.001	0.428	0.012	0.172	0.001
SBP	0.207	<0.001	0.685	<0.001	0.121	0.023
DBP	0.231	<0.001	0.419	0.014	0.176	0.001
Pulse	0.010	0.849	-0.011	0.951	0.002	0.970

CCT: Cranial computed tomography, GCS: Glasgow Coma Scale, NIHSS: National Institute of Health Sciences Scale, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, ONST: Optic nerve sheath thickness

In this study, a significant correlation between ONST; and age, HT, DM, AF, CAD, previous CVA and COPD/asthma ($p > 0.05$) was not detected. Also, it was determined that the ONST diameter increased with comorbidities and CHF ($p < 0.05$).

The hospitalization unit (clinic/ICU) was not found to be correlated with ONST ($p > 0.05$), however, the ONST values of the patients that died were significantly higher ($p < 0,05$) (Table 4).

A ROC curve was prepared to demonstrate the effectiveness of ONST in indicating ICP. The area under the curve has been detected to be 0.865; 4.5 mm cut-off value for 96.1%, specificity was 82.7% sensitivity (Figure 2).

Table 4. The correlation between ONST and comorbidities, hospitalization unit and death.

Parameters	Group 1	Group 2	P
Gender	5.25 (0.25)	5.25 (0.25)	0.664
Comorbidity	5.25 (0.25)	5.20 (0.21)	0.039
HT	5.25 (0.30)	5.20 (0.25)	0.158
DM	5.25 (0.25)	5.25 (0.25)	0.619
AF	5.30 (0.30)	5.25 (0.25)	0.051
CAD	5.25 (0.21)	5.25 (0.25)	0.809
Previous CVA	5.30 (0.35)	5.25 (0.25)	0.088
COPD/Asthma	5.25 (0.42)	5.25 (0.25)	0.404
CHF	5.33 (0.34)	5.25 (0.25)	0.016
Other	5.52 (0.41)	5.25 (0.25)	0.001
Outcome	5.25 (0.25)	5.25 (0.25)	0.435
Death	5.45 (0.54)	5.25 (0.25)	0.011

(Group 1: male, present, clinic, died; Group 2: women, intensive care, alive) HT: Hypertension, DM: Diabetes Mellitus, CAD: Coronary Artery Disease, AF: Atrial Fibrillation, CVA: Cerebrovascular Accident, COPD: Chronic Obstructive Pulmonary Disease, CHF: Congestive Heart Failure.

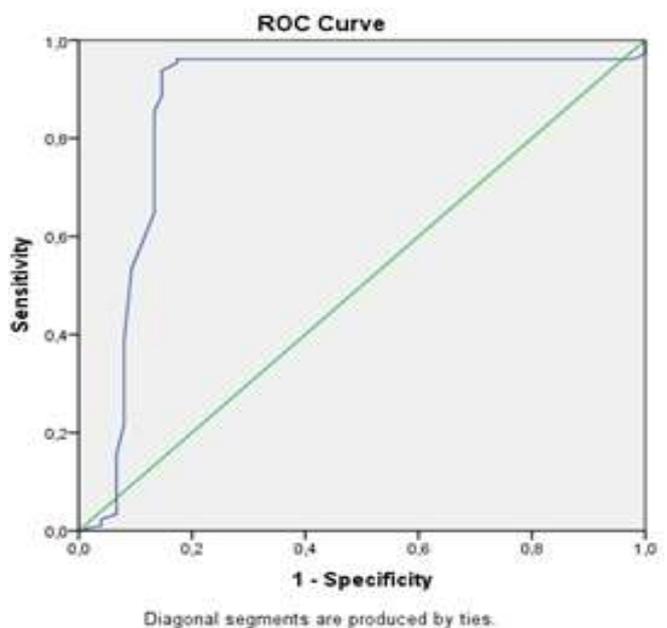


Figure 2. ONST diagnostic ROC curve.

Discussion

The increase in ICP due to intracranial events can lead to the deterioration of cerebral perfusion, increase in ischemia and brain tamponed. Thus, it is accompanied by high mortality and mortality rates. Brain edema can be detected in the earlier stages and treated with brain edema decompression, which can decrease mortality from 78% to 29% [11]. The increased intracranial pressure should be reduced as quickly as possible [12,13].

The relationship between ONST and ICP is contradictive. Kimberley et al. found a direct correlation between ONST and ICP [14]. Safak et al. reported that the diameter of ONST, measured through non-invasive methods, can be used to measure ICP [15]. However, it has been demonstrated that, in some special cases, the ONST doesn't increase despite increasing ICP. This is thought to be because the optic nerve sheath cannot expand in some patients, and due to the optic nerve sheath variations [16,17].

Komut et al. found that mean ONST diameter was 5.4 mm in the patients with stroke, whereas this value was 4.1 mm for the control group. Such difference was found to be significant [4]. Yuzbasioglu et al. [18] found the mean ONST diameter to be 5.6 mm for stroke patients; this value was 3.6 mm for the control group. This difference was found to be significant. Gokcen et al. [6] indicate that the ONST diameter was higher for the stroke patients compared to the control group. Skoloudik et al. [16] indicated that there wasn't a significant difference between the ONST diameters of stroke patients and healthy controls; however, the median ONST value was significantly higher among hemorrhagic stroke patients. In our study, we found that the ONST diameter of the stroke patients was significantly higher. We believe that the increase in the ONST diameter is due to

the primary events that occur due to hypoxia and the edema that develops due to the increased mediators.

Studies have found that 80-87% of all stroke cases are of is ischemic origin [2,3]. Chae et al. [19] indicate that the clinical condition of the hemorrhagic patients is worse compared to the ischemic stroke patients. Skoloudik et al. [16] have found that the ONST diameter was above 6.6 mm for 21% of the stroke patients, and above 5 mm for 71% of the patients. The same study found that the ONST diameter of 86% of hemorrhagic stroke patients was above 6.6 mm. Komut et al. [4] have found that the ONST diameter was higher for the hemorrhagic patients compared to the ischemic patients; however, this difference was not statistically significant. In our study, the incidence of hemorrhagic stroke was 7.4%, which is lower than the literature. It was also determined that the ONST diameter was higher for the hemorrhagic patients. This might be associated with the fact that; for patients who have hemorrhagic stroke, the bleeding can lead to more extensive damage, more significant disruption of the CSF circulation, and that there is more secondary damage.

Studies indicate that the patients with NIHSS and low GCS scores had higher mortality rates, and that these two scales can be used to predict clinical prognosis [20-22]. Komut et al. [4] found that the patients with lower GCS scores have higher ONST values. Yuzbasioglu et al. [20] found a positive correlation between NIHSS scores and ONST values. Jeng et al. [23] reported higher NIHSS scores and lower GCS scores among hemorrhagic stroke patients. In our study, we have found that ONST values were positively correlated with NIHSS scores and negatively correlated with GCS scores. It is known that increasing ICP worsens clinical condition. We believe that this

outcome decreases the GCS score while increasing the NIHSS. We believe this is the reason why ONST is positively correlated with NIHSS, whereas it is negatively correlated with GCS.

High rates of hypertension and arrhythmias are reported for stroke patients. This is explained by the alteration of the sympathetic and parasympathetic efferent pathways from the cardio regulator centers of the brain stem due to the stroke [24-26]. Achieving cerebral auto-regulation and decrease of cerebral parenchyma may cause an increase in the blood pressure [27]. Gokcen et al. [6] indicate that the stroke patients have elevated blood pressures. In our study, we found that the blood pressure and pulse values of the stroke patients had increased. A positive correlation was found between blood pressure and ONST; however, pulse rate was not found to be correlated with ONST. We believe that the impulses from the cardio-regulatory centers aim to increase the blood flow to the brain, thus increasing blood pressure and pulse. Since one of the components that determine ICP is blood pressure, we believe that a correlation occur between ONS and blood pressure. Furthermore, edema develops and blood pressure increases in proportion to hypoperfusion to prevent the hypoperfusion appeared due to irregular cerebral blood supply.

Studies indicate that comorbidities; such as DM, AF, HT, and previous SVO; may be risk factors for stroke, and that they increase the prevalence of strokes [23,28,29]. Complying with the literature, we have found the most common comorbidities in stroke patients to be HT (60.9%) and DM (30.9%); also, the prevalence of DM and AF had significantly increased for the patient group. In addition, ONST had significantly increased in patients with comorbidities and/or CHF. The most

common pathologies may be HT and DM because these diseases are also the most common comorbidities. DM may increase the prevalence of stroke due to the disruption of vascular structures, and AF leads to increased rates of heart-related thrombi.

In his dissertation, Batur [30] found that the rate of increased intracranial pressure syndrome (IICPS) was higher among the patients that were hospitalized in the intensive care unit compared to the patients hospitalized in the clinics; however, this rate was lower than the patients who died. Legrand et al. [31] found that ONST was smaller among patients that survived traumatic brain damage. In our study, the ONST values of the patients that were hospitalized or that died were higher. This may be due to the fact that increased ICP worsens the clinical condition of the patients, and the physician decides to hospitalize the patient with a poor condition to the ICU. Also, the patients with increased ICP values have higher mortality rates.

The dysfunction of auto-regulation in cerebral blood flow in acute stroke cases causes secondary edema increase [27]. In the present study, a positive correlation was detected between USS period and ONST. We believe that such correlation appears due to further dysfunction of auto-regulation and eventual increase of edema and ICP increase because earlier interventions cannot be performed to the patient before clarification of the diagnosis.

Dadi et al. [32] conducted a study on the children with findings of increase in ICP and reported a positive correlation between ONST measured by USS and ONST measured by brain CT and MRI. The aforementioned study also stated that such association was stronger in the measurements performed within first 5 hours. Sekhon et al. [33] reported a strong positive correlation between cranial CT and

ONST measured by USS in their study conducted on the cases with traumatic brain injury. In line with the literature, a strong correlation was detected between ONST detected by CT and USS in the present study. Batur [30] found in his dissertation that sensitivity of USG examinations was 95.7% for the IICPS-positive patients, whereas the specificity was 100%. Gokcen et al. [6] found that, for the 5.3 mm cut-off value, the sensitivity was 80% and the specificity was 84%. For the 4.7 mm cut-off value, the sensitivity was 70% and the specificity was 86%. In our study, we have found that for the 4.5 mm cut-off values, sensitivity and specificity were determined to be 96.1% and 82.7%, respectively. These values indicate that ONST can be a valuable parameter for the confirmation of the diagnosis of stroke patients and the indication of increased ICP.

The most important limitation of the present study is lack of control ONST measurements after follow-up and treatment. Further studies may be carried out on this topic. Another limitation is unclear time of the clinic presentation of the patient. This may be associated with unnoticeable clinical presentation at the beginning or attacks when the patients were alone or at sleep. However, many medical history data of the patients was not documented and added during history taking.

Conclusion

ONST values increase among stroke patients, and especially in patients with hemorrhagic strokes. If increase of ONST is detected in a patient, a treatment planning to reduce ICP may be considered.

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ORCID iD of the author(s)

Kaan Celik / 0000-0002-9664-6732

Tamer Colak / 0000-0003-3844-4785

Beliz Oztok Tekten / 0000-0002-4007-005X

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The efficacy of multiparametric prostate magnetic resonance imaging in the diagnosis and treatment of prostate cancer

Serkan Ozcan¹  · Yiğit Akin¹ · Osman Kose¹ · Mehmet Coskun² · Muhsin Engin Uluc² · Sacit Nuri Gorgel¹ · Yuksel Yilmaz¹ · Omer Faruk Yagli³

¹Department of Urology, İzmir Katip Çelebi University, School of Medicine, İzmir, Turkey

²Department of Radiology, İzmir Katip Çelebi University, School of Medicine, İzmir, Turkey

³Department of Urology, Tuzla State Hospital, İstanbul, Turkey

ABSTRACT

Aim: To investigate the accuracy of multiparametric prostate magnetic resonance imaging (mpMRI) in determining the diagnosis and treatment options of prostate cancer (PCa), and its pathology correlation.

Methods: Between October 2017 and January 2018, 73 patients were subjected to an mpMRI at our clinic. Of these patients, 11 were radical prostatectomy (RP) after treatment, and four were post-radiation therapy (RT) follow-up. The remaining 58 patients were assigned to the PSA elevation and / or positive digital rectal examination (DRE) patient group in this study and their outcomes were evaluated.

Results: Of the 58 patients included in the study, 13 were found to have a PI-RADS 5 on mpMRI and in 9 (90%) of 10 patients undergoing simultaneous biopsy, PCa was detected. The biopsy results of all cases evaluated as PI-RADS 1 were benign. All of the patients who were ISUP 3 and above had a PI-RADS 5. Patients with a PI-RADS score of 4 and above being ISUP 2 and above was statistically significant ($p=0.011$). A case had undergone a previous radical prostatectomy assessment revealed that tPSA increased to 2 ng/ml during the follow-up, and so RT was added to the treatment; although LAP was identified in the left iliac region on an mpMRI performed upon the continued increase of tPSA. During the follow-ups of the patient who had regional RT, the tPSA dropped below 0.01 ng/ml.

Conclusion: The results of our study show that mpMRI can gain a new and important place in urology due to the guidance it provides in biopsies, facilitating targeted biopsy, its effectiveness in determining treatment modalities and its importance in post-PCa treatment follow-ups.

Keywords: Multiparametric prostate magnetic resonance imaging, mpMRI, prostate cancer, PI-RAD.

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✉ Dr. Serkan Ozcan,
Department of Urology, İzmir Katip Çelebi University,
School of Medicine, İzmir, Turkey
E-mail: drserkanozcan@hotmail.com

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Introduction

Prostate cancer (PCa) is the second most common cancer among men in the United States of America (USA) and the second-leading cause of cancer-related deaths [1, 2]. The leading risk factor for prostate cancer is age, with the average age of diagnosis of PCa being 66 [3].

Although prostate cancer is common, the mortality risk is low. At this point, a risk classification has been made in order to answer the “Which PCa is fatal?” question. The National Comprehensive Cancer Network (NCCN) guidelines identifies six different risk groups, being very low, low, intermediate, high, very high and metastatic. This classification is based on “whether the cancer is limited to the prostate, the Gleason score, the number of specimens with cancer, the prostate-specific antigen (PSA) value, PSA density (PSAd) and the presence of metastasis to lymph nodes or other organs” [4, 5].

PSA and digital rectal examinations (DRE) are the current screening methods, with a biopsy recommended in cases where $PSA \geq 4$ ng/ml or a suspicious exam finding is present. The systematic biopsy of the prostate involves the use of a thick needle to take specimens of the peripheral zone, in line with certain standards. At this point, two basic issues need to be taken into account, the first of which is the failure to diagnose cancers in the areas that cannot be accessed by the needle due to the random sampling of cancers that cannot be viewed using ultrasonography (US), and the second issue is the over-diagnosis of low grade cancer. In this sense, an important weakness has emerged in PCa imaging. With the increased clinical use of 3 Tesla (T) devices, PCa can be viewed with a high accuracy rate, which has led to the development of multiparametric prostate magnetic resonance imaging (mpMRI) [6, 7].

An mpMRI is usually performed to identify localized cancers, along with elevated PSA. An mpMRI is aimed mainly at identifying clinically significant cancers (CSC: a tumor > 0.5 cc, Gleason $\geq 3+4$, extracapsular extension) [8], although some make use of mpMRI as the primary screening method [9].

The current guidelines differ in their recommendations on the use of mpMRI. The European Association of Urology (EAU) identifies two main strategies for mpMRI prior to biopsy: The first involves performing a systematic biopsy in all cases, regardless of the mpMRI result (positive or negative), and to add a targeted biopsy in the presence of a positive mpMRI; while second one involves only a targeted biopsy in the presence of a positive mpMRI, with no biopsy recommended in the event of a negative mpMRI. The EAU guidelines also point out that mpMRI is safer prior to a repeated biopsy [10].

Additionally, mpMRI is recommended if there is any clinical suspicion of PCa prior to the biopsy, and that every lesion identified should be biopsied in a targeted and systematic way [10]. According to NCCN guidelines, mpMRI should be considered in the active follow-up group with a life expectancy of more than 10 years in the very low- and low-risk groups. In cases where the biopsy is negative, yet a clinical suspicion still exists, mpMRI should be considered to allow observation of the anterior tumor in particular. mpMRI has also been recommended in the presence of elevated PSA in treated cases [5]. mpMRI has the same diagnostic power as computed tomography (CT) in identifying the pelvic pathological lymph node [11, 12]. mpMRI is also superior to bone scintigraphy and direct radiography in determining bone metastasis [13].

In the light of the above information, the present study assesses the accuracy of mpMRI

in the diagnosis and the determination of treatment options in PCa, and its pathology correlation.

Materials and Methods

A total of 73 patients underwent an mpMRI at our clinic between October 2017 and January 2018, of which 11 were radical prostatectomy (RP) cases and four were post-RT follow-up cases.

Patient exclusion criteria were standard MR contraindications and any previous prostate specific treatment (hormonal therapy, radiotherapy, or radical prostatectomy). Since no prostate imaging, reporting and data system (PI-RADS) categorization was made among the cases that had undergone treatment, so 15 cases were excluded from the study. The remaining 58 patients were assigned to the PSA elevation and/or positive DRE patient group in the present study, and their outcomes were evaluated. PI-RADS assessments were made prospectively, and then the mpMRI results of the cases were compared with their pathology results. The study was conducted in accordance with the ethical approval of the University Ethics Committee (Number: 47104536-000-8728). The rights of all participants were protected and written informed consents were obtained before the study according to the Helsinki Declaration.

Transrectal ultrasonography-guided biopsies

Biopsies were performed from 12 quadrants with a length of 15–22 mm by the guidance of a transrectal probe using a biopsy gun (Geotek® Estacore). 18 gauge needles were used. All patients were given antibiotic prophylaxis with ciprofloxacin before the procedure and bowel preparation was performed with an enema on the day of the procedure. The first dose was taken 1 day prior to biopsy and the second dose on the morning

of the biopsy. The antibiotic prophylaxis was continued for 1–3 days after biopsy. Rectal swab culture or targeted antibiotic therapy was not performed as a standard prior to the biopsies.

Multiparametric prostate magnetic resonance imaging (mpMRI)

Multiparametric prostate MRI comprises three basic sequences to achieve anatomical and functional imaging [8]. The first sequence is T2-weighted (T2A) imaging with a high spatial resolution, which allows for the differentiation between structures, such as the transitional zone (TZ), peripheral zone (PZ), capsule, pseudocapsule and urethra, providing anatomical detail. The second basic sequence is diffusion-weighted imaging (DWI), which incorporates two different images: high b-valued images ($b=0, 200, 800$ and 1400 sec/mm^2) and an apparent diffusion coefficient (ADC) map. DWI basically provides the image of the motion of water. Water moves freely in every direction in the extracellular space in normal prostate tissue, meaning that it displays an accelerated diffusion. In cases of increased cellularity and impaired tissue microarchitecture, the water cannot move freely in every direction, meaning that it displays a restricted diffusion. This manifests as a high signal on high b-value images and a low signal on the ADB map in DWI. The third basic sequence is dynamic contrast-enhanced (DCE) imaging, which provides details on tissue perfusion (in DCE, an at least 2-minute image is obtained in total every 15 seconds following the intravenous administration of a contrast agent. This allows information to be obtained on how fast the tissue gets blood, and how much, and how much of the blood it retains) [14].

PI-RADS v2 scoring

The scoring (categorization) was made based

on the recommended PI-RADS v.2 guidelines, which advise some dominant sequence scoring. Accordingly, peripheral zone (PZ) lesions are categorized based on the DWI score, while the transitional zone (TZ) lesions are based on the T2 score.

Located in PZ, the linear or wedge lesions that are slightly low on ADC and isointense on high b-value image are categorized as score 2; those intermediately low on ADC and with a slightly high signal on high b-value image are categorized as score 3; those that are prominently low on ADC with a high signal on a high b-value image and <15 mm are categorized as score 4; those ≥ 15 mm with characteristics of a score 4 signal or lesions with an extraprostatic extension are categorized as score 5; and those with score 3 and early focal contrast involvement on DCE are categorized as score 3+1=4.

Located in the TZ, lesions that are regular, encapsulated and nodular are categorized as score 2; those with heterogeneous signals and irregular contours are categorized as score 3; those that are homogeneous, hypointense and limited to the prostate and <15 mm are categorized as score 4; and those measuring ≥ 15 mm with characteristics of a score 4 signal, or lesions with extraprostatic extensions, categorized as score 5 [8].

ISUP classification

Today, pathology reports are required to include a grade classification, from 1 to 5, in addition to the Gleason score assignment for PCa [15]. Such classifications are made based on the guidelines for prostate cancer, which are graded in accordance with the scale identified at a consensus conference organized in 2014 by the International Society of Urological Pathology (ISUP). Upon the recommendations of the 2014 consensus conference, the 2005 ISUP classification has been changed (Table 1).

Table 1. The International Society of Urological Pathology (ISUP) grading system.

ISUP grade	Gleason scores	Definition
Grade 1	2–6	Only individual discrete well-formed glands
Grade 2	3+4=7	Predominantly well-formed glands with lesser component of poorly formed/fused/ cribriform glands
Grade 3	4+3=7	Predominantly poorly formed/fused/ cribriform glands with lesser component of well-formed glands
Grade 4	4+4=8	Only poorly formed/fused/cribriform glands
	3+5=8	Predominantly well-formed glands and lesser component lacking glands (or with necrosis)
	5+3=8	Predominantly lacking glands (or with necrosis) and lesser component of well-formed glands
Grade 5	9–10	Lacking gland formation (or with necrosis) with or without poorly formed/fused/ cribriform glands

Statistical analysis

Data analysis was performed with SPSS software, version 22 for Windows. Numerical parameters were expressed as mean \pm standard deviation, minimum and maximum values, while categorical variables were expressed as frequency and percentage. The Mann-Whitney U test for nonparametric data was used to determine the significance of differences between the groups. $P < 0.05$ was considered to be statistically significant.

Results

Of the 58 patients included in the study, 13 were found to have a PI-RADS score of 5 on an mpMRI, 10 of which underwent concurrent biopsy and 9 (90%) were identified as having PCa (One of them; Figure 1). Based on the biopsy results of the group with a PI-RADS score of 4, 71% were diagnosed with PCa, although 11 of the 20 patients evaluated as PI-RADS 1 underwent a biopsy, and all were found to be benign (Table 2).

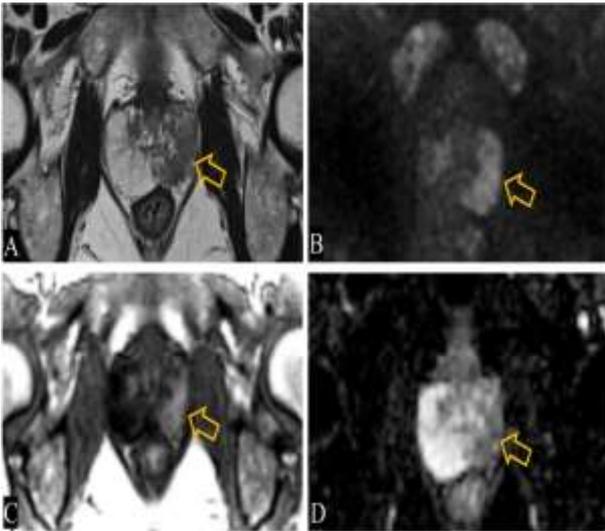


Figure 1. The left mid peripheral zone lesion (yellow arrows) was hypointense on T2WI (a), hyperintense on high b-value (b). It was vividly enhancing in early arterial dynamic imaging (c) and hypointense on ADC (d). This was a PI-RADS category 5 lesion with a 31 mm diameter. It was diagnosed Gleason 4+3 after radical prostatectomy.

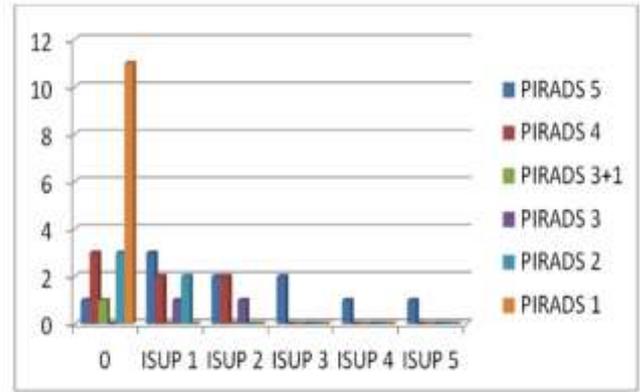


Figure 2. The PI-RADS distribution according to the ISUP classification (58 cases).

surgical contour was negative and no metastasis was identified, and therefore RT was administered. An mpMRI performed upon the continued increase of tPSA during the follow-up identified a 15x9 mm LAP in the left iliac region (Figure 3). During the follow-ups of the patient who had regional RT thereafter, the tPSA dropped below 0.01 ng/ml.

Table 2. The PI-RADS and ISUP results of 58 cases with PI-RADS scoring.

Scoring	Patients (n)	0	ISUP 1	ISUP 2	ISUP 3	ISUP 4	ISUP 5	Non-Biopsy (n)	PSA	PSA D
PI-RADS 5	13	1	3	2	2	1	1	3	19 (4,6-124,91)	0,409
PI-RADS 4	10	2	3	2	0	0	0	3	6,79 (3,25-12,16)	0,159
PI-RADS 3+1	3	1	0	0	0	0	0	2	3,53 (1,99-5,23)	0,067
PI-RADS 3	4	0	1	1	0	0	0	2	4,55 (1,09-7,63)	0,098
PI-RADS 2	8	3	2	0	0	0	0	3	7,62 (2,12-18,74)	0,109
PI-RADS 1	20	11	0	0	0	0	0	9	5,69 (0,34-13,79)	0,072

All of the patients who were 3 and above according to the ISUP classification had a PI-RADS category of 5 (Figures 2). Patients with a PI-RADS score of 4 and above being ISUP 2 and above (CSC) was statistically significant ($p=0.011$).

The additional case assessment revealed that tPSA increased to 2 ng/ml during the follow-up of one patient with a tPSA=9.94 ng/ml in 2014 who was diagnosed with Gleason =3+4 upon the systematic biopsy, and who had undergone a previous radical prostatectomy, although the

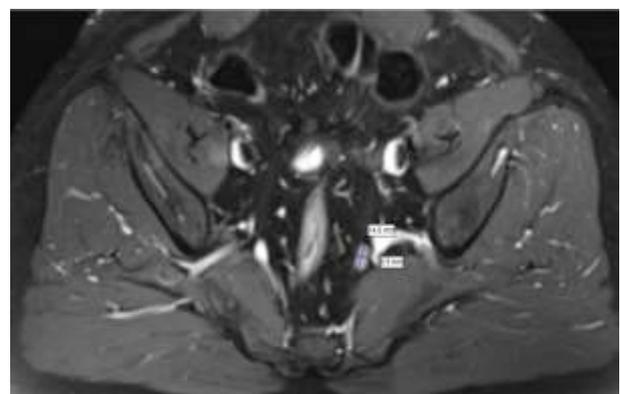


Figure 3. 15x9-mm left main iliac LAP on contrasted T1A on mpMRI.

Discussion

Prostate MRI was first used to evaluate extraprostatic invasion during PCa staging. Combining different sequences of MR imaging, mpMRI has gained in popularity in recent years, and has started to be used to guide TRUS biopsies for PCa diagnosis [16]. The significance of using mpMRI, especially prior to a prostate biopsy, was also emphasized in the 2012 European Society of Uroradiology Guidelines [17].

In a review of the success of mpMRI, a study with 3T reported that CSC was detected in 99 of 100 patients [18]. In another study, involving 114 patients with no lesions identified on mpMRI, found identified no lesions in a systematic biopsy in 88 (77.2%) cases, while a Gleason 3+3 tumor and a Gleason 3+4 tumor was identified in 22 (19.3%) and 4 (3.6%) cases, respectively. The success in ruling out CSC was found to be 96.5%. All of the Gleason 3+4 cases were observed to be patients with active follow-ups [19]. Another study also reported a very high rate of negative prediction for mpMRI (97–98.7%) [20].

In light of the updated knowledge, a positive mpMRI (PI-RADS score of 4 or 5) allows a targeted biopsy to be performed in patients who have not undergone a biopsy, but who have an elevated PSA and/or positive DRE. With the negative mpMRI (PI-RADS score 1 or 2), the biopsy can be delayed and a PSA follow-up can be considered. A negative mpMRI has a very high success rate in ruling out CSC. A positive mpMRI can reveal anterior tumors or CSC in the region that cannot be accessed by a biopsy needle in patients with a negative biopsy history, despite an elevated PSA. A negative mpMRI, on the other hand, can reveal the causes of elevated PSA, such as prostatitis, an enlarged prostate gland and BPH nodules. In patients with a positive biopsy history, a

positive mpMRI can detect extracapsular extension, seminal vesicular invasion and neurovascular bundle invasion. This changes the treatment strategy (extended surgery or higher-dose radiotherapy rather than neuroprotective surgery). An active follow-up may be considered in the presence of a negative or minimal abnormal mpMRI, a low tumor volume, a Gleason 3+3 score or a short life expectancy (the NCC recommends monitoring only in the very low, low and intermediate risk groups, and with a life expectancy lower than 10 years). However, mpMRI may not reveal high-risk cancer in some cases, and therefore careful PSA monitoring should be carried out during the active follow-up. In patients with a post-treatment elevated PSA, a positive mpMRI can display a recurrence, leading to early treatment. Again, these patients require close follow-up in the presence of a negative mpMRI, as in such cases, a systemic disease may be present [9].

In the present study, the identification of PCa in most of the cases with PI-RADS 4 or 5, and in groups of patients with pathologies of ISUP 2 or more, allows for patient prediction prior to biopsy. Furthermore, it increased the rate CSC identification, and helped in the differentiation of a patient group that might require active follow-up. The benign biopsy result in all of the patients biopsied among the cases evaluated with PI-RADS 1 suggests that a biopsy may be avoided in patient groups recording such results. The study by Wang et al., which is in line with the present study, also reported reduced unnecessary diagnosis for the low-grade cancer group, and avoided repeated biopsies through the performance of targeted biopsies [21].

mpMRI plays a significant role also in local assessment following prostate cancer treatment. An mpMRI following a radical prostatectomy,

RT and focal treatment may be used to visualize normal post-treatment changes and to detect recurrent diseases locally [22]. In the additional case assessment provided in our study, the tPSA of one patient who underwent a radical prostatectomy had increased, although the surgical contour was negative and no metastasis was identified during follow-ups, and the patient received RT. However, an mpMRI performed after a continued increase of tPSA was identified during follow-up revealed a LAP in the left iliac region. A regional RT was administered and tPSA dropped below 0.01 ng/ml during the follow-up, which supports its usefulness in viewing recurrences.

This study has some limitations. Firstly, it is retrospectively designed. Secondly, our sample number is a little low, but it is acceptable for a pilot study. Also, the mpMRI has started to be applied in the near future.

It can be understood from the findings of the present study that mpMRI has gained a novel and significant place in urology in providing guidance to biopsies, in allowing targeted biopsies to be performed, in aiding in the determination of treatment modalities and in its significant contributions to post-PCa treatment follow-ups. That said, the number of participants in our study needs to be increased in order to reflect the general population, and so further studies are required.

Conclusion

MpMRI has gained a novel and significant place in urology in providing guidance to biopsies, in allowing targeted biopsies to be performed, in aiding in the determination of treatment modalities and in its significant contributions to post-PCa treatment follow-ups. Additionally, mpMRI can also be considered an appropriate imaging method for revealing localized tumors in cases with recurrent PSA.

Our study observed that a targeted biopsy is required at the diagnostic stage, and the PI-RADS classification is an important indicator of biopsy.

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ORCID iD of the author(s)

Serkan Ozcan / 0000-0002-2459-139X

Yiğit Akin / 0000-0001-7627-3476

Osman Kose / 0000-0003-4070-6676

Mehmet Coskun / 0000-0003-4339-898X

Muhsin Engin Uluc / 0000-0002-1919-1797

Sacit Nuri Gorgel / 0000-0001-7628-1249

Yuksel Yilmaz / 0000-0002-0548-9322

Omer Faruk Yagli / 0000-0002-6248-4421

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Effect of foot anthropometric measurements on postural stability



Seda Sertel Meyvaci¹ · Turgut Meyvaci² · Rengin Kosif¹ · Murat Diramali¹ · Handan Ankarali³

¹Department of Anatomy, Bolu Abant İzzet Baysal University, Faculty of Medicine, Bolu, Turkey

²Family Medicine Physician, Semerkant Family Health Center, Bolu, Turkey

³Department of Biostatistics and Medical Informatics, Istanbul Medeniyet University, Faculty of Medicine, Istanbul, Turkey

ABSTRACT

Aim: To examine the effect of foot anthropometric measurements and body sizes of young male adults with normal posture on balance.

Methods: In this study, the effect of body size and foot anthropometric measurements of 112 young male adults with normal posture on balance was investigated. The foot and body parameters of the cases were measured. The static and the dynamic balance tests were evaluated according to the dominant foot in each case. The parameters that affected balance were determined and the variables were taken to the model. In addition, the significance levels that defined the effects of the properties examined in relation with the balance were also calculated.

Results: When the findings were evaluated, it was determined that the effect of the foot parameters other than the foot length, and the effect of 15 body parameters other than the biiliac diameter, trochanteric height, and right upper extremity length on balance performance was significant. The balance test performance was predicted with success ranging from 7.8% to 43% with the parameters included in the model.

Conclusion: In this study, the fact that the relation between the foot anthropometric and body dimensions and functional balance performances of young male adults was found to be significant shows that this relation must be considered in the creation of a normative database on balance, and in clinical studies that will be conducted on the subject.

Keywords: Foot, postural balance, anthropometry, body measures, young adult, male.

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✉ Seda Sertel Meyvaci,

Department of Anatomy, Bolu Abant İzzet Baysal University, Faculty of Medicine, Bolu, Turkey

E-mail: sedasertelmeyvaci@gmail.com

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Introduction

As a measurement technique in the human body, anthropometry can be defined as a

quantitative expression technique of the shape of the human body [1].

Today, anthropometry is considered as the most portable, universally applicable, cheap and noninvasive technique to evaluate the size, proportions and composition of the human body [2]. With the help of this technique, especially individual differences like age and gender in the human body, and population

differences variables were evaluated in previous studies [3,4].

Anthropometric assessments in the human body contains the measurements of circumferences, height, diameter, and fat mass tissue. When the studies in the literature were examined, many studies using these anthropometric measurement methods were identified [5,6]. However, the individual anthropometric differences that were assessed in these studies, which evaluated mostly body components and positions, were limited with height, weight and Body Mass Index (BMI), and did not assess other anthropometric changes [7,8].

It is necessary to evaluate the results of studies according to anthropometric differences, because there will be changes in individual body components even if the study is limited with height, weight and BMI in the planning of the previous studies.

In the present study, the purpose was to examine the effect of foot anthropometric measurements and body sizes of young male adults with normal posture on balance.

Materials and Methods

The participants of the study were selected randomly from among the volunteers who were educated at or who worked at Bolu Abant İzzet Baysal University, and those who fit the inclusion criteria were included in the study. A total of 112 volunteering men that had an 18-25 age range and a BMI in normal limits were included in the study.

Considering the gender and age selection of the participants, the different hormonal cycle of every woman, difficult to standardize, which can cause changes in the musculoskeletal system affecting our balance performance results, only young male cases were included in our study, and their characteristics were evaluated.

The selection criteria for the participants were:

Inclusion criteria: Not having neurological or orthopedic disease that might cause balance disorder. Not having foot deformity. Not have any past surgery that might affect the foot and musculoskeletal system.

Having normal posture and asymptomatic status.

Having BMI within normal limits (18.50-24.9 kg/m²).

Being between the ages of 18 and 25.

Having male gender.

Not having sports-doing history.

Being volunteer to take part in the study.

Exclusion criteria: Having a neurological or orthopedic disease that might cause balance disorder. Having foot deformity. Having past surgery that might affect the foot and musculoskeletal system.

Not having normal posture and asymptomatic status.

Not having the BMI scores within the normal limits.

Being between the ages of 18 and 25.

Having female gender.

Having sports-doing history.

Not volunteering to participate in the study.

This study was conducted in accordance with the rules of the Declaration of Helsinki. Written informed consent was obtained from each participant. The rights of the subjects were protected. It was approved by the Bolu Abant İzzet Baysal University Clinical Researches Ethics Committee Approval, Decision No:2018/91.

Measurements

In the present study, the parameters and balance tests of the foot and body dimensions of the cases were evaluated. In addition, posture evaluation of the cases were examined by using

posture analysis by visual observation method. To determine the parameters of the foot, body dimensions and the deformity status of the cases, the measurements and evaluations were made by using a digital caliper, digital goniometer, height meter, length meter, tape measure ruler, and foot-graphics device. All the measurements were performed by the same evaluator and balance performance tests were performed under doctor's supervision. The measurements were recorded in millimeters (mm) or degrees. In addition, for each case, age, height (stature), weight, BMI, dominant hand, dominant foot and all measurements were recorded in Excel.

Foot Parameters

The names and abbreviations of the foot parameters that were measured are given below. All measurements were made with a Digital Caliper [9-11].

1. Foot Length (FL)
2. Foot Width (FW)
3. Foot Heel Width (FHW)
4. Foot Height (FH)
5. Medial Malleoli Height (FMH)
6. Lateral Malleoli Height (FLH)
7. Height of Metatarsophalangeal Joint at First Toe (FM1)
8. Height of Metatarsophalangeal Joint at Fifth Toe (FM5)
9. Instep Apex Height (FAH)
10. Navicular Height (FNH)

Body Parameters

The names and abbreviations of the 18 body parameters that were measured are given below [11-13].

1. Right Upper Extremity Length (RUL)
2. Left Upper Extremity Length (LUL)
3. Lower Extremity Length (LL)
4. Subtalar Joint Angle (SJA)
5. Feet Opening Angle (OA)
6. Acromial Height (AH)
7. Trochanteric Height (TH)

8. Patellar Height (PH)
9. Trunk Length (TrL)
10. Thigh Length (ThL)
11. Shank Length (SL)
12. Biacromial Diameter (BAD)
13. Biiliac Diameter (BID)
14. Bitrochanteric Diameter (BTD)
15. Bimalleolar Diameter (BMD)
16. Chest Circumference (CC)
17. Waist Circumference (WC)
18. Hips Circumference (HC)

Balance Tests

In the present study, each case was tested for functional reach, flamingo balance test, which is a static balance test, and time-up and go (TUG), which is a dynamic balance test, 10 meter walk (10 M), and Y-balance test; and the results were evaluated according to the dominant foot. In the TUG and 10 M test, the participants were asked to walk at the highest speed in a previously measured limited area.

1. **Flamingo Balance Test:** The test was applied for both lower extremities. The results were evaluated according to the dominant (FB) and non-dominant (FB-N) side [14].
2. **Y Balance Test:** The test was applied to both lower extremities. The anterior, medial, lateral components of the test were evaluated according to dominant (YA, YM, YL) and non-dominant (YA-N, YM-N, YL-N) side [15,16].
3. **Time-up and Go Test (TUG):** Since it is a performance to which both lower extremities take part in, it was not evaluated according to dominant side [17].
4. **10-meter Walk Test (10 M):** Since it is a performance to which both lower extremities take part in, it was not evaluated according to dominant side [18].
5. **Functional Reach Test:** The test was applied to the right (RFR) and left (LFR) upper extremities [19].

Statistical Analysis

The descriptive statistics of the data were calculated as mean, Standard Deviation (SD) and quartile values. Multiple linear regression model with forward selection methods was used for the determination of the effects of body sizes and foot anthropometric measures on balance. Statistically significant level was accepted as $P < 0.05$.

Results

The descriptive statistical values of the numerical variables of the cases included in the evaluations were examined, and the demographic characteristics of them were determined (Table 1).

When we examined all the cases in terms of dominant foot, the dominant foot of 96 cases among the 112 cases was identified as the right

Table 1. The demographic characteristics of the cases.

Demographic Characteristics	N	Mean	Standard Deviation	Percentiles		
				25	50	75
Age (years)	112	20.31	1.95	19.00	20.00	21.75
Height (cm)	112	176.09	6.13	172.00	176.35	179.95
Weight (kg)	112	70.97	7.85	64.77	71.10	75.99
BMI (kg/m ²)	112	22.85	1.78	21.24	23.03	24.54

Table 2. The descriptive values of the dominant and non-dominant foot of the cases.

Foot Parameters (mm)	Dominance Status	N	Mean	Standard Deviation	Percentiles		
					25	50	75
FL	dominant	112	26.30	1.27	25.60	26.09	27.32
	non-dominant	112	26.38	1.23	25.60	26.35	27.22
FW	dominant	112	11.35	0.91	10.06	10.37	10.84
	non-dominant	112	10.51	0.55	10.12	10.54	10.94
FHW	dominant	112	6.27	0.48	5.98	6.31	6.62
	non-dominant	112	6.35	0.49	6.03	6.34	6.67
FMH	dominant	112	8.44	0.63	8.00	8.45	8.90
	non-dominant	112	8.45	0.62	8.00	8.50	9.00
FLH	dominant	112	7.13	0.66	6.80	7.20	7.50
	non-dominant	112	7.16	0.57	6.73	7.15	7.50
FM1	dominant	112	3.33	0.41	3.10	3.30	3.50
	non-dominant	112	3.34	1.02	3.10	3.20	3.40
FM5	dominant	112	2.24	0.54	2.10	2.20	2.30
	non-dominant	112	2.24	0.55	2.00	2.20	2.30
FAH	dominant	112	7.15	0.49	6.80	7.10	7.50
	non-dominant	112	7.10	0.50	6.73	7.05	7.50
FNH	dominant	112	4.83	0.67	4.40	4.90	5.20
	non-dominant	112	4.77	0.68	4.30	4.80	5.20
FH	right side	112	7.12	0.54	6.73	7.20	7.50

foot, and the dominant foot of 16 cases was identified as the left foot. The descriptive values of the foot parameters were assessed as dominant foot and non-dominant foot. The FH parameter was measured from one place, on the right side of the case (Table 2).

The descriptive values of all the cases except for the feet were assessed, and are given in Table 3. The descriptive values of the static and dynamic balance test measurements of all the cases were evaluated according to the dominance status of the lower extremities (Table 4).

We examined the parameters that affected static balance tests. It was determined that FM1 and FAH, which are the foot parameters, together with LL, SJA and OA body parameters had

effects on the dominant side in the FH balance test ($P<0.05$). Since the P value of the foot FMH parameter was $P<0.10$ in the resulting model, it was left in the model because it would affect the model significantly when it was discarded from the model. The effect of this variable was also important on balance. When the degree of importance of the parameters that were included in the model in predicting the FH test was examined, it was determined that the FM1 parameter, which had the highest importance, had the highest effect on FH balance. The FH balance test is estimated with 25.9% success with the 6 parameters included in this model (Table 5). When all the systemic and anatomical variables in the body that are effective on balance were considered, it

Table 3. The descriptive values of the body anthropometric measurements of the cases.

Body anthropometric measurements	N	Mean	Standard Deviation	Percentiles		
				25	50	75
RUL	112	78.59	3.71	76.48	78.65	80.88
LUL	112	78.53	3.69	76.05	78.75	80.70
LL	112	91.93	4.52	89.35	91.50	94.95
SJA	112	2.15	1.08	1.00	2.00	3.00
OA	112	19.09	5.65	14.96	18.38	22.80
AH	112	144.91	5.43	141.85	144.50	147.88
TH	112	91.09	6.53	88.35	91.50	94.15
PH	112	49.62	6.12	48.00	49.95	51.40
TrL	112	53.08	3.63	51.00	53.40	55.50
ThL	112	42.02	3.34	40.00	41.85	44.08
SL	112	43.44	5.97	41.08	43.00	44.30
BAD	112	40.58	1.60	39.70	40.49	41.60
BID	112	28.93	2.05	27.68	29.17	30.18
BTD	112	33.18	1.82	32.23	33.22	34.27
BMD	112	7.45	1.11	7.05	7.29	7.61
CC	112	91.16	5.35	88.00	91.00	95.00
WC	112	77.72	7.13	74.85	78.00	82.45
HC	112	94.84	7.88	92.00	95.50	99.00

becomes important to reach this high predictive value as a result of measuring the 6 parameters. It was determined that the foot parameter FM1 and PH, SJA and OA body parameters had effect on the FH-N balance parameter ($P<0.05$). Since the P value of the foot FAH parameter was <0.10 in the resulting model, it was left in the model because it would affect the model significantly when it was discarded from the model. The effect of this variable was also important on balance. When the degree of importance of the parameters that were included in the model in predicting the FH-N test was examined, it was determined that the FM1 parameter, which had the highest importance, had the highest effect on FH-N balance. The FH-N balance test is estimated with 20.6% success with the 5 parameters included in this model (Table 6).

The foot and body parameter of the Y balance test, which is one of the dynamic balance tests, affecting the YA performance, could not be determined, in other words, there are no parameters in this model to predict YA ($R^2=0\%$). Only the FW affects the YM balance,

and it was also determined that the CC parameter might have a significant effect on the model ($P<0.10$). The YM balance test is estimated with 7.8% success with the 2 parameters included in this model.

When the YL balance was examined, it was found that it only affects the FW. It was also found that SJA and FHW might have an effect on the model ($P<0.10$). The YL balance test is estimated with 10.5% success with the 3 parameters included in this model (Table 7). When the Y balance test, which is the other balance test, was examined, it was determined in the non-dominant extremity that it was determined that the foot parameters, FMH and FW, which affected YA-N performance, and CC and HC from the body parameters. It was determined that the FLH, FM5, OA, ThL and BMI parameters would affect the YA-N balance test results significantly in the resulting model (Table 8). When the degree of importance of the parameters that were included in the model in predicting the YA-N test was examined, the CC parameter, which had the highest importance, had the highest

Table 4. The descriptive values of the static and dynamic balance test measurements of the cases.

Balance Tests	N	Mean	Standard Deviation	Percentiles			
				25	50	75	
FH	112	7.79	5.47	3.25	7.00	11.75	
FH-N	112	7.68	5.51	3.00	7.00	11.00	
Y Balance	YA	112	83.37	10.85	75.47	83.65	90.31
	YM	112	60.44	12.24	51.90	60.71	68.58
	YL	112	72.89	14.69	63.52	72.47	84.26
	YA-N	112	82.90	13.40	75.76	83.10	90.41
	YM-N	112	62.99	10.77	55.86	63.69	69.87
	YL-N	112	74.53	12.60	64.09	74.41	85.09
TUG	112	5.37	0.96	4.70	5.17	6.11	
10 M	112	5.23	0.67	4.71	5.21	5.76	
RFR	112	27.65	8.84	20.72	28.45	34.09	
LFR	112	26.37	8.45	19.33	27.19	32.28	

effect on the YA-N balance. The YA-N balance test is estimated with 21.2% success with the 9 parameters included in this model.

Only FW, which is among the foot parameters, and the SL, CC and BMI, which are among the body parameters, affect the YM-N balance. It was determined that the FM5 parameter has a significant effect on the model ($P < 0.10$). When the degree of importance of the parameters that were included in the model in predicting the YM-N test was examined, the CC parameter, which has the highest significance, has the highest effect on the YM-N balance. The YM-N balance test is estimated with 15.0% success

with the 5 parameters included in this model.

When the YL-N balance was examined, it was determined that only the FW, which is among the foot parameters, and the CC, SL, SJA and BMI, which are among the body parameters, affect it. It was determined that only ThL might have an effect on the model ($P < 0.10$). When the degree of importance in predicting the YL-N test was examined, it was determined that the CC parameter, which had the highest significance, had the highest effect on the YL-N balance. The YL-N balance test is estimated with 14.6% success with the 6 parameters included in this model (Table 8).

Table 5. The effect of foot and body parameters on the FH test.

Model term FH	Coefficient	SD±	t	P	95% Confidence interval		Importance
					Lower	Upper	
Intercept	-13.531	9.836	-1.376	0.172	-33.034	5.972	
FM1	6.715	1.701	3.948	0.0001	3.342	10.087	0.329
FAH	-4.278	1.348	-3.172	0.002	-6.952	-1.604	0.213
LL	0.453	0.171	2.647	0.009	0.114	0.793	0.148
SJA	-1.109	0.438	-2.532	0.013	-1.978	-0.241	0.135
OA	-0.182	0.083	-2.189	0.031	-0.346	-0.017	0.101
FMH	1.899	1.016	1.869	0.064	-0.116	3.914	0.074

Table 6. The effect of foot and body parameters on the FH-N test.

Model term FH-N	Coefficient	SD±	t	P	95% Confidence interval		Importance
					Lower	Upper	
Intercept	-20.218	10.314	-1.960	0.053	-40.666	0.230	
FM1	5.807	1.744	3.330	0.001	2.350	9.265	0.327
PH	0.584	0.183	3.194	0.002	0.221	0.946	0.300
SJA	-1.032	0.460	-2.244	0.027	-1.944	-0.120	0.148
OA	-0.177	0.084	-2.100	0.038	-0.344	-0.010	0.130
FAH	-2.065	1.149	-1.797	0.075	-4.342	0.213	0.095

Table 7. The effect of foot and body parameters on the Y balance test.

Model term Y Balance		Coefficien t	±SD	t	P	95% Confidence interval		Importanc e
						Lower	Upper	
YM	Intercept	-23.205	25.142	-0.923	0.358	-73.036	26.625	
	FW	4.870	1.886	2.582	0.011	1.132	8.608	0.699
	CC	0.361	0.213	1.696	0.093	-0.061	0.782	0.301
YL	Intercept	37.262	26.073	1.429	0.156	-14.418	88.942	
	FW	7.073	2.261	3.128	0.002	2.591	11.556	0.585
	SJA	-2.368	1.268	-1.868	0.064	-4.881	0.145	0.208
	FHW	-5.265	2.829	-1.861	0.065	-10.872	0.343	0.207

Table 8. The effect of foot and body parameters on the Y-N balance test.

Model term Y-N Balance		Coefficient	±SD	t	P	95% Confidence interval		Importance
						Lower	Upper	
YA-N	Intercept	93.833	30.976	3.029	0.003	32.393	155.274	
	CC	1.217	0.345	3.523	0.001	0.532	1.902	0.236
	FMH	-8.552	2.603	-3.285	0.001	-13.715	-3.389	0.205
	HC	-0.842	0.339	-2.484	0.015	-1.515	-0.170	0.117
	FW	5.188	2.205	2.353	0.021	0.814	9.563	0.105
	FLH	5.378	2.744	1.960	0.053	-0.064	10.820	0.073
	OA	-0.412	0.213	-1.935	0.056	-0.833	0.010	0.071
	BMI	-2.182	1.137	-1.919	0.058	-4.436	0.073	0.070
	ThL	-0.683	0.379	-1.802	0.075	-1.434	0.069	0.062
FM5	11.129	6.296	1.768	0.080	-1.359	23.617	0.059	
YM-N	Intercept	-8.455	25.752	-0.328	0.743	-59.512	42.601	
	CC	0.849	0.277	3.068	0.003	0.300	1.398	0.327
	FW	4.637	1.726	2.687	0.008	1.216	8.058	0.251
	BMI	-1.872	0.840	-2.228	0.028	-3.538	-0.206	0.172
	SL	-0.730	0.362	-2.016	0.046	-1.449	-0.012	0.141
	FM5	9.029	5.100	1.770	0.080	-1.083	19.141	0.109
YL-N	Intercept	51.095	29.546	1.729	0.087	-7.490	109.679	
	CC	1.022	0.334	3.062	0.003	0.360	1.683	0.254
	FW	5.704	1.953	2.920	0.004	1.830	9.577	0.231
	BMI	-2.508	0.997	-2.516	0.013	-4.485	-0.531	0.171
	SL	-1.010	0.430	-2.350	0.021	-1.863	-0.158	0.149
	SJA	-2.348	1.072	-2.191	0.031	-4.474	-0.223	0.130
	ThL	-0.558	0.360	-1.550	0.124	-1.272	0.156	0.065

When the TUG balance was examined, only the PH, LL and BAD, which are among the body parameters, and FHW, which is among the foot parameters, affect it. It was also determined that BTD, ThL, TrL and SJA might have an effect on the model ($P < 0.10$). When the degree of importance of the parameters that were included in the model in predicting the TUG test was examined, it was determined that the PH parameter, which had the highest importance, had the highest effect on the TUG balance. The TUG balance test is estimated with 32.0% success with 8 parameters included in this model (Table 9).

When the 10 M balance was evaluated, it was determined that only the FNH, which is among foot parameters, affected it, and no body parameters affected it. It was also determined that FM5, FHW and BAD might have effect on the model ($P < 0.10$). When the significance level of the parameters that were included in the model in predicting the 10 M test was examined, it was determined that the FNH parameter, which had the highest significance level, had the highest effect on the 10 M balance. The 10 M balance test is estimated with 15.0% success with the 4 parameters included in this model (Table 10).

Table 9. The effect of foot and body parameters on the TUG balance test.

Model term TUG	Coefficient	±SD	t	P	95% Confidence interval		Importance
					Lower	Upper	
Intercept	6.369	2.496	2.552	0.012	1.418	11.319	
PH	0.136	0.039	3.522	0.001	0.060	0.213	0.274
LL	-0.090	0.027	-3.327	0.001	-0.143	-0.036	0.244
BAD	-0.118	0.054	-2.189	0.031	-0.225	-0.011	0.106
FHW	0.346	0.171	2.028	0.045	0.008	0.685	0.091
BTD	0.095	0.051	1.876	0.064	-0.005	0.195	0.078
ThL	0.047	0.026	1.791	0.076	-0.005	0.098	0.071
SJA	0.134	0.076	1.774	0.079	-0.016	0.285	0.070
TrL	-0.043	0.025	-1.740	0.085	-0.092	0.006	0.067

Table 10. The effect of foot and body parameters on the 10 M test.

Model term 10 M	Coefficient	SD±	t	P	95% Confidence interval		Importance
					Lower	Upper	
Intercept	6.637	1.756	3.781	0.0001	3.157	10.118	
FNH	0.248	0.096	2.586	0.011	0.058	0.438	0.421
FM5	-0.587	0.309	-1.902	0.060	-1.200	0.025	0.228
BAD	-0.066	0.038	-1.727	0.087	-0.141	0.010	0.188
FHW	0.216	0.134	1.615	0.109	-0.049	0.481	0.164

When the RFR test was examined, the effects of FNH and FAH, which are among foot parameters, and LUL, LL and OA, which are among the body parameters, were determined. The effect of BTD was not found to be at statistically significant level on the model. When the significance level of the parameters that were included in the model on predicting the RFR test was examined, it was determined that the LUL parameter, which had the highest significance level, had the highest effect on the RFR balance. The RFR balance test is estimated with 27.0% success with the 6 parameters included in this model (Table 11).

When the LFR test was evaluated, it was determined that the AH, CC, LUL, BTD, BAD, WC ve BMI, which are among the body parameters, and the FNH, FW ve FH, which are among the foot parameters, had effects. When the significance level of the parameters that were included in the model in predicting the LFR test was examined, it was determined that the FNH parameter, which had the highest significance level, had the highest effect on the LFR balance. The LFR balance test is estimated with 43.0% success with the 10 parameters included in this model (Table 12).

Table 11. The effect of foot and body parameters on the RFR test.

Model term RFR	Coefficient	SD±	t	P	95% Confidence interval		Importance
					Lower	Upper	
Intercept	48.488	18.893	2.566	0.012	11.027	85.950	
LUL	-0.839	0.282	-2.975	0.004	-1.399	-0.280	0.239
FNH	-3.941	1.419	-2.777	0.006	-6.754	-1.127	0.208
LL	0.659	0.245	2.696	0.008	0.174	1.144	0.196
OA	0.334	0.136	2.466	0.015	0.065	0.603	0.164
FAH	3.669	1.821	2.014	0.047	0.058	7.280	0.110
BTD	0.877	0.499	-1.758	0.082	-1.867	0.112	0.083

Table 12. The effect of foot and body parameters on the LFR test.

Model term LFR	Coefficient	SD±	t	P	95% Confidence interval		Importance
					Lower	Upper	
Intercept	9.001	21.085	0.427	0.670	-32.831	50.832	
FNH	-6.451	1.228	-5.253	0.0001	-8.887	-4.014	0.203
AH	1.160	0.225	5.150	0.0001	0.713	1.607	0.195
BMI	3.071	0.712	4.312	0.0001	1.658	4.483	0.137
CC	-0.714	0.203	-3.513	0.001	-1.117	-0.311	0.091
LUL	-0.829	0.240	-3.459	0.001	-1.304	-0.353	0.088
FW	-3.615	1.171	-3.087	0.003	-5.938	-1.292	0.070
BTD	-1.371	0.486	-2.822	0.006	-2.336	-0.407	0.059
FH	4.240	1.589	2.668	0.009	1.087	7.393	0.052
BAD	1.214	0.483	2.515	0.014	0.256	2.172	0.047
WC	-0.438	0.202	-2.171	0.032	-0.839	-0.038	0.035

When all of the systemic and anatomic variables of the body that are effective on balance were evaluated, it was determined that measuring these parameters is important in having high predictive value in studies that are based on performance like balance and in achieving accurate results.

Discussion

Anthropometric data provides information regarding the static dimensions of the human body in standard postures. Anthropometry has been used as the indicator of health status in national anthropometric sizing studies over years [20]. Knowing all the factors that might affect anthropometric measurements is important for accurate planning of studies, by considering individual anthropometric differences and obtaining accurate results in studies, and more data are necessary. These anthropometric measurements are affected by many factors which include age, height, weight, gender and ethnic origin. For this reason, many studies were conducted by considering the factors, which affect anthropometric measurements [21-23].

When the studies in the literature were examined, it was determined that many researchers considered the factors, which affected anthropometric measurements, and which limited these factors, especially age and gender, to obtain more accurate results [24-26]. Many researchers who are interested in anthropometric measurements conducted studies to standardize these values in their population by considering the racial differences and ethnicity [27].

In the present study, many anthropometric measurements of the foot and body were examined by limiting age, BMI and gender.

Many researchers who are interested in foot anthropometry conducted studies on foot

measurements, shoe dimensions, foot print, plantar pressure, sex identification, age and stature estimation [28-31]. In these studies, most of the measurements were made with the help of calipers and measuring tapes in addition to radiography and computed tomography [9,32-34].

In the present study, which examined the effects of foot anthropometric measurements on balance, the foot parameters were measured bilaterally by using digital caliper.

When similarly-planned studies were examined, it was determined that some researchers examined the parameters like FL [12,34,35], FW [10,12,34], FHW [9,36], FH, FMH, FLH, FM1, FM5, FAH ve FNH [9,10,28], which are among foot parameters, were investigated by some researchers. When the results were compared, it was determined that although some results were similar to ours, some other results were different. We believe that this might be due to the fact that researchers included different number of cases in their studies, and that the cases did not have the same age and population.

When the studies conducted on body parameters were examined, it was determined that stature, weight, upper and lower extremity lengths; body heights like AH, TH, PH, and lengths of body parts like TrL, ThL, SL; diameter and circumference measurements, foot and hand measurements; anthropometric measurements like OA, SJA and BMI were evaluated by researchers [10,11,13,27,37]. As in these studies, in our study, body heights like stature, weight, RUL, LUL, LL, AH, TH, PH, height of body parts like TrL, ThL, SL, circumference measurements like CC, WC, HC, foot measurements and OA, SJA, BMI parameters were evaluated.

Among the studies, which necessitate that the physical participation of individuals like

balance, which may affect the results, the studies assessing balance are important.

When other studies conducted in the fields of running, swimming and other sports that require balance-level performance were examined, many body anthropometric parameters were taken into account [7,37-39]. It was found that there are limited studies especially examining the relations between balance and foot and body anthropometric measurements [40,41]. In their study, Alonso et al. evaluated the balance parameters with the help of a device, and found that there was a relation among height, trunk-cephalic length, upper-limb length, and lower-limb length [40]. In their study, Keionen et al. examined the effect of foot parameters on balance, and concluded that there was a relation among height, hip-ground distance, and knee-ground distance and some balance parameters especially in foot width, heel width, and foot length [41]. In another study conducted by Moein and Movaseghi on female cases, no relation was detected about foot length parameter, although a relation was detected with the lower leg length parameter and balance [42]. In the present study, the effect of the foot parameters on balance was examined, together with other body parameters, and what effect it had on a performance like balance was investigated. When the findings were evaluated, it was determined that the other foot parameters except for the foot length, the effect of the body parameters except for the length of the right upper extremity, biiliac diameter, and trochanteric height had effects on balance performance. The foot width (0.699) had the highest significance among the foot parameters, and the chest circumference (0.327) among the body parameters had the highest significance. It was concluded that balance test performance can be predicted with the parameters included

in the model with a success ranging from 7.8% to 43%. In the present study, it was also found that especially BMI, chest, waist and hip circumference measurements had effects on the results of some balance tests.

Conclusions

Balance is defined as the ability to keeping the center of gravity of the body on the support center connected to a good functional postural control system. This complex nervous system process is fed with visual, auditory and somatosensorial stimuli. The resulting answer is a whole of neuromuscular stimulation reaching the musculoskeletal system [43].

Balance is controlled with the detection of the movements, positions and proprioceptive senses coming from the foot by adjusting them according to the environment with the central nervous system. As a result of our study, it was determined that the anthropometric of the foot and body had an effect on the balance results.

In the present study, which included only male cases and in which the age range was limited to 18-25, normative data of balance were obtained and contributed to the literature by considering all the factors that might affect balance performance.

Which among the different foot and body parameters would affect static and dynamic functional balance tests was also determined in our study. In this respect, different parameters should be considered according to the test to be performed when selecting balance tests.

In the light of the obtained data in the present study, it was found that the relation between foot anthropometric and body measurements and functional balance performances of young male adults with normal posture is significant; and we believe that the results can be useful in evaluating and planning of future clinical studies.

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ORCID iD of the author(s)

Seda Sertel Meyvaci / 0000-0002-9450-145X

Turgut Meyvaci / 0000-0002-8836-1956

Rengin Kosif / 0000-0002-0334-1658

Murat Diramali / 0000-0001-5824-6159

Handan Ankarali / 0000-0002-3613-0523

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The effects of meteorological factors and air pollution on prognosis of idiopathic sudden sensorineural hearing loss

Elif Karali ·  Akif Gunes

Department of Otorhinolaryngology, Bolu Abant Izzet Baysal University, Medical Faculty, Bolu, Turkey

ABSTRACT

Aim: To evaluate the effect of air pollution parameters and meteorological factors on the prognosis of idiopathic sudden sensorineural hearing loss (ISSNHL).

Methods: 40 patients diagnosed with ISSNHL who were treated in our clinic between 2015 and 2018 were examined retrospectively. Meteorological data including average temperature (T_{mean}), maximum and minimum temperature (T_{max} and T_{min}), relative humidity, and air pollution parameters including sulfur dioxide (SO₂) and particulate matter (PM 10). Data of 10 days prior to the disease and 14 days after the treatment were analyzed.

Results: When the distribution of patients according to the seasons were examined, it was found that 12 (30%) of the patients were seen in autumn, 11 (27.5%) in spring, 9 (22.5%) in winter, and 8 (20%) in summer. When the T_{max} , T_{min} , T_{mean} values obtained as of the initiation of the treatment were compared, it was found that the values of the group without recovery were significantly lower. Relative humidity values were significantly lower in the group without recovery in pre-treatment and post-treatment measurements. No significant difference was found between the recovery groups in SO₂ and PM10 values in pre-treatment and post-treatment measurements.

Conclusion: It was observed that relative humidity, T_{max} , T_{min} , T_{mean} values may affect prognosis in ISSNHL patients in our study. In addition, SO₂ and PM10 were not associated with ISSNHL recovery rates. Our study is the first in the literature in terms of evaluating the relationship between air pollution parameters and ISSNHL prognosis.

Keywords: Sudden sensorineural hearing loss, meteorological factors, air pollution, particulate matter.

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✉ Dr. Elif Karali,

Department of Otorhinolaryngology,
Bolu Abant Izzet Baysal University, Medical Faculty,
Bolu, Turkey

E-mail: elifkarali8181@gmail.com

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Introduction

Sudden hearing loss (SHL) which is an emergency case developing within 72 hours is defined as at least 30dB sensorineural hearing loss at three consecutive frequencies. Although there are many hypotheses for its etiology such as viral, vascular and autoimmune pathologies, etiopathogenesis of SHL remains unclear [1,2].

Since viral infections are considered one of the important etiological factors of idiopathic sudden sensorineural hearing loss (ISSHL), it is believed that meteorological conditions may affect the initiation and prognosis of ISSHL. Weather changes have been shown to be involved in the pathogenesis of various diseases by suppressing the immune system [3,4]. The results of the studies in the literature investigating the relationship between weather conditions and the development of ISSHL are different [5–8].

The increasing air pollution around the world in parallel with global urbanization and industrialization reveals concerns about its negative effects on health. Particulate matter (PM) is a common air pollutant consisting of a mixture of solid and liquid particles suspended in the air. Particulate matter with a diameter of $\leq 10 \mu\text{m}$ (PM10) is the most harmful to human health among all air pollutants [9–11]. It has been reported that inhaled endotoxins in PM can contribute significantly to the induction of respiratory inflammation and dysfunction [12]. Epidemiological studies have shown a relationship between cardiovascular and respiratory diseases and atmospheric PM10 and SO₂ levels [13,14]. Vascular disorders are also considered to have an important role in the pathogenesis of SSNHL. ISSHNL has been shown to be associated with an increased incidence of cardiovascular disease [15,16]. This relationship suggests that air pollutants may play a role in the pathogenesis of ISSNHL. Studies evaluating the effect of meteorological factors on the prognosis of ISSNHL are limited and their results are controversial. There are no previous studies investigating the effects of air pollution parameters on the prognosis of ISSNHL. The aim of this study is to evaluate the effect of air pollution parameters and meteorological factors on the prognosis of

idiopathic sudden sensorineural hearing loss (ISSNHL).

Materials and Methods

In this retrospective study, the files of patients diagnosed with idiopathic sudden sensorineural hearing loss in our clinic between 2015 and 2018 were examined. Patients who received a single treatment protocol, who started the treatment within 1 week of the disease and who did not start the treatment at another center were included in the study. Patients having an unclear specific initiation date, previous autological surgery, cerebellopontine angle pathology, chronic otitis media, Meniere's disease, hypertension and diabetes were excluded from the study. A standard treatment protocol was applied to all patients. (250mg i.v. methylprednisolone on the first day followed by 1mg/kg/day i.v. methylprednisolone for a total of 14 days, decreasing the dose by 20 mg every 3 days).

The study protocol was approved by the local ethics committee (Number: 2019/136) and the study was conducted in accordance with the principles of the Helsinki Declaration.

Audiological evaluation

Standard audiometric evaluation including 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz and 6 kHz thresholds was performed on all patients. ISSNHL patients were divided into groups according to the Siegel's criteria recommended by the American Academy of Otolaryngology-Head and Neck Surgery (3). Complete recovery was classified as having a final pure tone average (PTA) of less than 25 dB. Partial recovery was classified as recovery of more than 15 db in PTA and having a final PTA of 25-45 dB. Mild recovery was classified as recovery of more than 15 db in PTA and having a final PTA of >45 dB. No recovery was

classified as recovery of less than 15 db in PTA and having a final PTA of >75 dB.

Meteorological data

Meteorological data was obtained from the web-based national air quality monitoring network. All patients were living in the same region having the same climatic conditions. 4 meteorological parameters mean temperature (T_{mean}), maximum temperature (T_{max}), minimum temperature (T_{min}), average relative humidity) and 2 air pollution parameters (sulfur dioxide (SO2) and PM10) were controlled 1-7 days before ISSNHL and 14 days after ISSNHL. The relationship between the mean value of the parameters before and after onset of ISSNHL and recovery rates were examined.

Statistical analyses

For the descriptive statistics, numerical variables were expressed as mean ± standard deviation or median [minimum maximum], and categorical variables were expressed as number and percentage. The normality assumption was examined with the Shapiro-Wilks test. Kruskal-Wallis test was used for comparing groups, considering the number of observations. In case of a difference, paired comparison test was used to determine the group/groups causing the difference. Regarding the analysis of the changes over time, Paired t-test was used when parametric test assumptions were met. Wilcoxon test was used in case of it was not available. The relationship between categorical variables was examined by Chi square test. $P < 0.05$ was accepted as statistically significant in all the analyses. Analyses were performed using IBM SPSS v.21.

Results

28 were male (30%) and 12 were female of the patients in the study. The mean age was $49.5 \pm$

14.53 years. Hearing loss was unilateral in all patients (62.5% right and 37.5 left). Pure tone average before treatment was 57.33 ± 21.62 . When the distribution of patients was examined according to the seasons, it was found that 12 (30%) of the patients were seen in autumn, 11 (27.5%) in spring, 9 (22.5%) in winter, and 8 (20%) in summer. According to the Shigel criteria, 16 (40%) patients had full recovery, 7 (17.5%) patients had partial/mild recovery, and 17 (42.5%) patients had no recovery (Table 1).

Table 1. Demographic and clinical data of the patients.

Parameters		N	%
Sex	Female	12	30.0
	Male	28	70.0
Seasons	Spring	11	27.5
	Summer	8	20.0
	Autumn	12	30.0
	Winter	9	22.5
Side	Left	15	37.5
	Right	25	62.5
Recovery	Complete recovery	16	40.0
	Partial / Mild Recovery	7	17.5
	Recovery	17	42.5
	No recovery		
Age (year) (mean ± SD)		49.5 ± 14.53	
Initial PTA (db) (mean ± SD)		57.33± 21.62	

SD: standard deviation PTA: pure tone averages

The mean age of those with full recovery was significantly lower ($P = 0.005$). When the first PTO was evaluated, it was significantly lower in the full recovery group ($P = 0.001$) (Table 2).

Table 2. Relationship between demographic, clinical features and recovery rates in patients with ISSNHL.

Parameters		Recovery rates			
		Complete recovery	Partial / Might Recovery	No recovery	<i>P</i>
Sex	Female	5 (41,7%)	1 (8,3%)	6 (50,0)	0.555
	Male	11 (39,3 %)	6 (21,4%)	11 (39,3)	
Side	Left	6 (40,0%)	3 (20,0%)	6 (40,0%)	0.942
	Right	10 (40,0%)	4 (16,0%)	11 (44,4%)	
Age (year)		41.5 ^a [19 - 72]	60 ^b [55 - 76]	52 ^{a,b} [23 - 71]	0.005
Initial PTA (db)		44.5 ^a [21 - 68]	77 ^b [46 - 112]	57 ^b [36 - 102]	0.001

^{a,b} indicates statistical differences of groups.

Table 3. Mean values of meteorological and air pollution parameters and difference analysis results based on Siegel’s criteria.

Parameters		Recovery rate			<i>P</i>
		Complete recovery (%)	Partial / Might recovery (%)	No recovery (%)	
TMax(°C)	Before	16.8 [5.71 - 29]	24.85 [10.4 – 35.2]	12.28 [1.57 – 35.2]	0.083
	After	15.75 [6.46 - 25] ^{a,b}	26.13 [9.13 - 30] ^a	9.53 [1.33 – 30.4] ^b	0.034
	<i>p</i>	0.438	0.866	0.469	
T Min(°C)	Before	4.71 [-3.2 – 15.25]	11.11 [0.57 – 15.7]	3.85 [-9 – 15.42]	0.164
	After	5.34 [0.33 – 10.4] ^{a,b}	12.8 [1.4 – 13.66] ^a	2.73 [-5 – 15.6] ^b	0.030
	<i>p</i>	0.959	0.735	0.653	
Tmean(°C)	Before	10.55 [1.26 – 22.13]	17.98 [6.05 – 25.45]	8.07 [-1.57 – 24.7]	0.118
	After	10.61 [3.4 – 17.7] ^{a,b}	19.89 [5.27 – 21.42] ^a	5.77 [-1.84 – 22.9] ^b	0.041
	<i>p</i>	0.679	0.866	0.687	
RH	Before	0.39 [0.13 – 0.77] ^{a,b}	0.56 [0.25 – 0.75] ^a	0.27 [0 – 0.72] ^b	0.018
	After	0.36 [0.14 – 0.72] ^{a,b}	0.66 [0.24 – 0.81] ^a	0.24 [0.02 – 0.74] ^b	0.013
	<i>p</i>	0.816	0.128	0.602	
SO2 (ppm)	Before	7.14 [3.5 – 42.5]	5.47 [2.4 – 15.96]	8.1 [3.6 – 29.36]	0.248
	After	6.3 [4.1 – 24.81]	6.1 [2.47 – 14.6]	7.9 [4.2 – 31.92]	0.221
	<i>p</i>	0.816	0.310	0.421	
PM 10 (µg/m ³)	Before	45.35 [20.3 – 100.5]	25.1 [18.5 – 74.3]	32.6 [17.3 – 100.2]	0.374
	After	57.4 [19.7 - 96]	26.6 [17.6 – 68.2]	50.2 [14.4 – 98.7]	0.110
	<i>p</i>	0.918	0.398	0.124	

T min: minimum temperature; T mean: mean temperature; T max: maximum temperature RH: Relative humidity. SO2: sulfur dioxide PM: particulate matter. ^{a,b} indicates statistical differences of groups.

There was no difference in T_{mean} , T_{max} and T_{min} values between the recovery groups in terms of data before the onset of the disease. When the data after initiation of the treatment were compared, it was found that the values of the group without recovery were significantly lower ($P < 0.005$). Relative humidity values among the recovery groups were significantly lower in the group without recovery in pre-measurements and post-measurements ($P < 0.005$) (Table 3). There was no significant difference between the recovery groups in terms of SO₂ and PM₁₀ values both in pre-measurement and post-measurements ($P > 0.005$) (Table 3).

Discussion

It has been revealed that the mean values of meteorological values T_{max} , T_{min} and T_{mean} and relative humidity before the onset of the disease and during the 14 day period after the initiation of treatment had an effect on the recovery rates. There was no relationship found between air pollution parameters SO₂ and PM₁₀ and ISSNHL recovery rates.

Various prognostic factors such as age, degree of hearing loss and time between hearing loss and the initiation of treatment, hypertension, and diabetes have been reported in the literature in patients with ISSNHL [17]. In the present study, patients who started the treatment within 1 week from the onset of the disease and who did not have hypertension and diabetes were included. The mean age and baseline pure tone average of those who fully recovered according to Shigel's criteria was significantly lower. These results were in consistency with the data in the literature.

The effects of weather conditions on the onset of ISSNHL have been investigated in previous studies [8,18–21]. Some researchers reported a significant relationship between the onset of

ISSHL and weather conditions [5,6,19], while others reported no relationship between weather conditions and the development of ISSNHL [7,8]. There are limited studies investigating the relationship between ISSNHL prognosis and meteorological factors [7,22]. The relationship between the daily temperature range and the recovery rates of sudden hearing loss remains unclear. Narozny et al. [17] reported the occurrence of this disease in spring as a positive prognostic factor. Durmuş et al. [22] reported a relationship between the average temperature and rainfall before the onset of ISSNHL and the prognosis of ISSNHL. In their study, Ryu et al. [7] evaluated the average temperatures from meteorological parameters and reported that the average temperatures at the onset of ISSNHL were not associated with the recovery rates. It was observed that the mean relative humidity values before and after the onset of ISSNHL and the average temperatures after the initiation of treatment were associated with the prognosis of ISSNHL. This is the first study to present that relative humidity before and after treatment is effective on prognosis.

There are various studies in the literature investigating the relationship between air pollution parameters (especially PM) and various diseases in recent years. It is reported that the increase in PM and decrease in humidity have increased hospitalization rates due to respiratory diseases in children and the elderly [23,24].

There are limited studies in the literature evaluating the relationship between air pollution parameters and ISSNHL [25,26]. Lee et al. [25] reported that there is a statistically significant but weak correlation between the number of patients hospitalized with the diagnosis of ISSNHL and the average daily PM value. Choi et al. [26] investigated the

relationship between air pollution parameters and ISSNHL and found that only concentrations of NO₂ were associated with ISSNHL. The present study is the first study evaluating the relationship between air pollution parameters and prognosis of ISSNHL. In the present study, there was no significant difference in the air pollution parameters examined (SO₂ and PM₁₀ values) between the ISSNHL recovery groups.

This study has certain limitations. The most important of these is the low number of patients and investigation of a single region. Meteorological conditions and air pollution show regional differences. In addition, different statistical analyses are required excluding the interaction between meteorological factors. Multicenter studies with large case series involving several regions and different climatic conditions are needed to clarify the relationship between meteorological factors and air pollution parameters and the prognosis of ISSNHL.

Conclusion

It was found in the study that relative humidity, T_{max} , T_{min} , and T_{mean} values may affect prognosis in ISSNHL patients. In addition, SO₂ and PM₁₀, two air pollution parameters, were not associated with ISSNHL recovery rates.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical statement: The study was conducted in accordance with the ethical approval of the

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ORCID iD of the author(s)

Elif Karali /0000-0002-8164-4056

Akif Gunes / 0000-0001-9554-8922

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increases the risk of SSNHL: A nested case-control study using meteorological data and national sample cohort data. *Sci Rep.* 2019;9(1):1–8.



A clinical review of autoinflammatory diseases and Behcet's disease: Classification, pathogenesis and treatment

Sengul Aksakal 

Department of Immunology and Allergy, Training and Research Hospital, Samsun, Turkey

ABSTRACT

Behcet's disease is a rheumatic disease with oral aphthae, genital aphthae, arthritis and vasculitis. Studies about its pathogenesis have increased and is thought to be one of the autoinflammatory diseases in recent years. Autoinflammatory diseases occurs via excess response of innate immune system. In this article pathogenesis and classification of autoinflammatory diseases will be summarized and Behcet's disease will be reviewed by autoinflammatory prospects.

Keywords: Autoinflammatory disease, Behcet's disease, innate immune system.

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✉ Dr. Sengul Aksakal

Department of Immunology and Allergy, Training and Research Hospital, Samsun, Turkey

E-mail: sengul.aksakal@saglik.gov.tr

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Introduction

Autoinflammatory diseases are episodic conditions that occur via excess response of innate immune system and cause fever and inflammation in many organs. Awareness of autoinflammatory diseases rose after definition of tumor necrosis receptor associated periodic syndrome (TRAPS) in 1999 by McDermott [1]. Advances in recognition of these diseases were developed in recent 20 years by increased awareness of this kind of conditions and by advances in genetic science. Treatment of these disease was advanced by therapies which target innate immune system. Autoinflammation is predominant in the pathogenesis of these conditions and may be together with

autoimmunity. Autoinflammation is different from autoimmunity by which the innate immune system is responsible from autoinflammation where B lymphocyte and other acquired immune system cells and cytokines are active in the latter. Therefore, disease specific antibody develops and be detected in autoimmunity, however, neither antibodies related to organ damage nor antigen specific t lymphocytes were detectable in autoinflammation [2]. Figure 1 shows these conditions and their pathogenesis.

Familial Mediterranean Fever (FMF) is the first autoinflammatory disease which was associated with genetics. Following this development, TNFRSF1 gene mutation was determined in TRAPS. Clinical findings of both diseases include episodic sterile inflammation, fever, myositis, arthralgia, rash-like rashes on the skin and serositis. The absence of any autoantibody and auto-reactive T cell positivity has led clinical research to focus on the innate

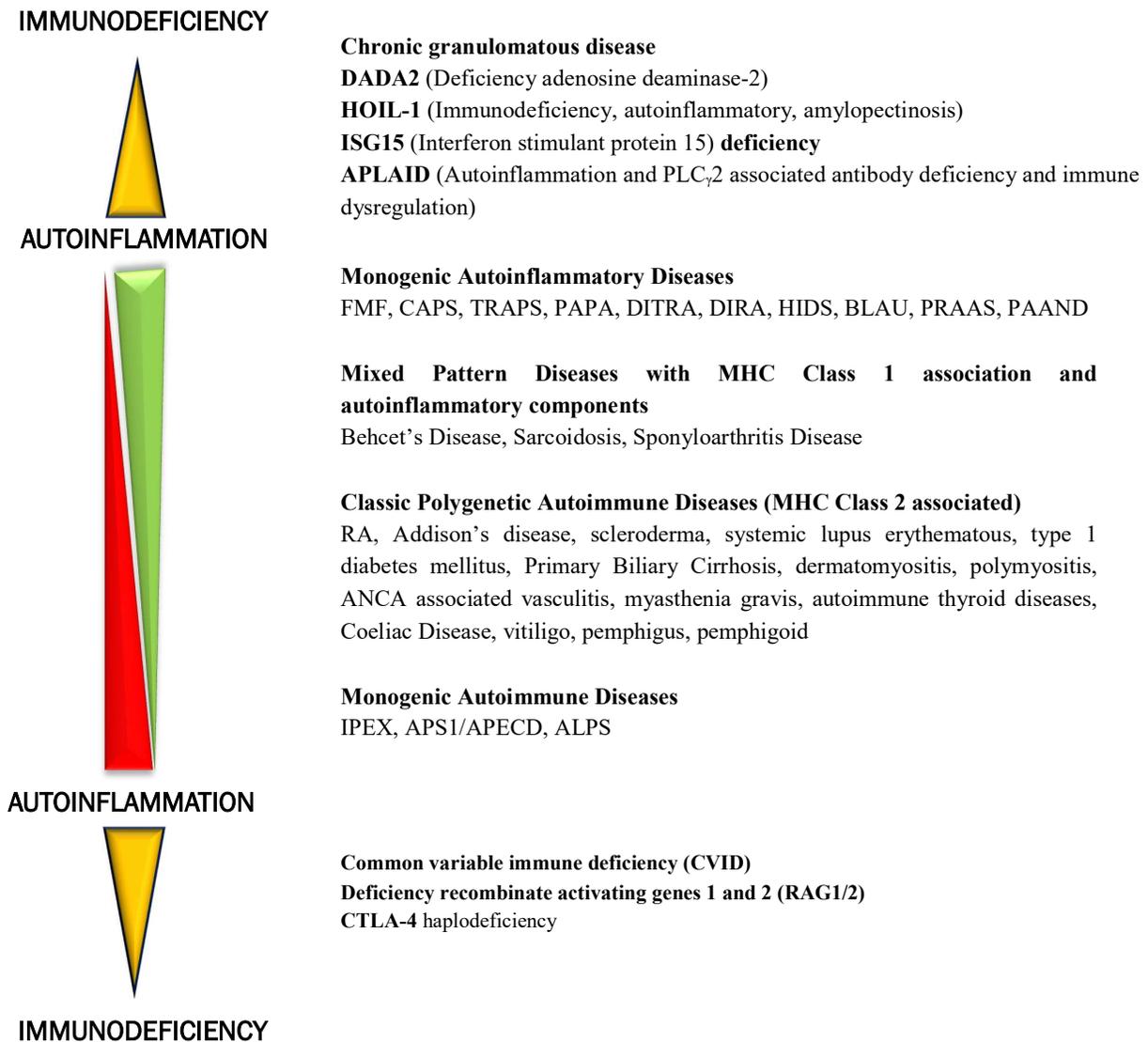


Figure 1. The spectrum of autoinflammatory diseases, FMF: Familial Mediterranean fever; TRAPS: Tumor necrosis factor receptor associated periodic syndrome; CAPS: Cryopyrin-associated periodic syndrome; HIDS: Hyperimmunoglobulin D Syndrome; PAPA: Pyogenic arthritis, pyoderma gangrenosum, acne; DIRA: Deficiency of the interleukin-1–receptor antagonist; PAAND: Pyrin associated autoinflammation with neutrophilic dermatosis; PRAAS: Proteasome-associated autoinflammatory syndromes; AOSD: Adult's still syndrome; R.A: Rheumatoid arthritis; ALPS: Autoimmune lymphoproliferative syndrome; APS-1: autoimmune polyendocrine syndrome type 1 of APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy); IPEX: immunodysregulation polyendocrinopathy enteropathy X-linked syndrome [2].

immune system and possibly related gene studies [3]. In present study, pathogenesis and classification of autoinflammatory diseases will be summarized and Behcet's disease will be reviewed by autoinflammatory prospects.

Pathogenesis of autoinflammatory diseases

The innate immune system is responsible of the first and fastest response to inflammation. This response is not antigen specific but consists of several cells and cytokines. The first cytokines

released as the innate immune system activated are interleukin-1 (IL-1), IL-8, tumor necrosis factor alpha (TNF α) and type 1 interferons (IFN α and IFN β).

Inflammasome

It is the signaling complex that provides the intracellular response of the innate immune system which was first described in 2002. Some cytosolic receptors in inflammasome formation include absent in melanoma-2 (AIM2), recombinant activation gene-1 (RAG-1), pyrin and most importantly NOD like receptor family (NLR) which includes NLRP3. It is schematized in Figure-2 [4].

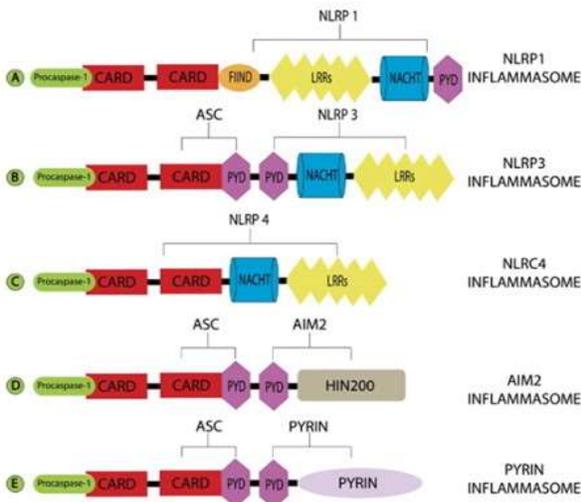


Figure 2. Inflammasome related cytoplasmic receptors [4].

In the stimulation of cytosolic receptors, apoptosis-associated speck-like protein-containing CARD (ASC) plays a role, acting as an adapter protein, cooperating in all inflammasomes. This step is organized by the inflammasome complex. It converts Procaspase-1, to the active form caspase-1 and stimulates the proinflammatory cytokines (pro-IL-1 β and pro-IL-18) to turn into their active forms. There are several factors that stimulate both endogenous and exogenous

inflammasomes. An example of exogenous stimulants is pathogen-related molecular patterns (PAMPs). Lipopolysaccharides in gram-negative bacteria cell wall, lipoteichoic acid in gram-positive bacteria, double-stranded RNA viruses, peptidoglycan, flagellin are some of the PAMPs. Endogenous factors include nucleus and cytosolic proteins, that occurred during cell-death and damage-associated molecular patterns (DAMPs), which reacts to potential cancer cells and remove cellular residues [3]. Activation of inflammasome is critical for the innate immune system, and genetic mutations at this stage cause uncontrolled activations, leading to autoinflammatory diseases.

Interleukin -1 β (IL - 1 β) and IL - 18

IL-1 β was detected before the identification of inflammasome. It is an acute phase reactant and a pyrogen. It is released from macrophages, dendritic cells, neutrophils and keratinocytes. It is activated by the IL-1 receptor type I (IL-1R) and stimulates the nuclear factor kappa light-chain-enhancer of activate B cell (NF κ B) and causes expression of cyclooxygenase-2. It also provides the release of IL-6 and TNF- α and cause fever. Under normal conditions, it is released during infections and initiates inflammation against the pathogens. Pathologically excessive stimulation or formation causes autoinflammation. Effects of IL-1 α plays a role via IL-1R in healthy individuals and has a very low importance in autoinflammation [6]. In contrast, inhibitory drugs developed against IL-1 β provided an important clinical response in the treatment of autoinflammatory diseases [7].

Information on IL-18 is less than IL-1 β . It plays a role as IFN- γ and proinflammatory cytokines. Its role in fever formation and as an acute phase reactant is weak. It has a role in inflammatory

Table 1. Monogenetic autoinflammatory diseases [10].

Monogenic autoinflammatory conditions	Gene (protein)	Clinic Manifestations	Treatment
Familial Mediterranean fever (FMF)	MEFV (pyrin)	Fever, abdominal pain, arthritis, erysipelas-like skin rashes	Colchicine, canakinumab, anakinra, rilonacept, TNF inhibitors
Cryopyrin-associated periodic syndromes (CAPS)	NLRP3 (cryopyrin)	Fever, conjunctivitis, arthritis, urticaria	Anakinra, rilonacept, canakinumab, steroid, NSAIDs
Mevalonate kinase deficiency (MKD)	MVK (mevalonate kinase)	Fever, severe abdominal pain, diarrhea, arthralgia, rash, lymphadenopathy, splenomegaly,	Corticosteroids, NSAIDs, anakinra, canakinumab, TNF inhibitors
Tumor necrosis factor receptor-associated periodic syndrome (TRAPS)	TNFRSF1A (TNF receptor type 1)	Fever, myalgia, abdominal pain, conjunctivitis	Corticosteroids, NSAIDs, canakinumab, anakinra, etanercept
Pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome	PSTPIP1 (proline/serine/threonine/ phosphatase-interacting protein)	Erosive arthritis, pyoderma gangrenosum, acne	Corticosteroids, anakinra, canakinumab, infliximab, adalimumab
Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND)	MEFV (pyrin)	Fever, arthralgia, myositis, neutrophilic dermatosis	Anakinra, infliximab, adalimumab
Blau's syndrome (familial juvenile systemic granulomatosis)	NOD2 (nucleotide-binding oligomerization domain-containing protein 2)	granulomatous reactions, tenosynovitis, uveitis	Corticosteroids, methotrexate, cyclosporin, TNF inhibitors, anakinra
Deficiency of the IL-1 receptor antagonist (DIRA)	IL1RN (IL-1 receptor antagonist)	Osteomyelitis, osteopenia, periostitis, pustular dermatitis	Anakinra, canakinumab, rilonacept
Deficiency of the IL-36 receptor antagonist (DITRA)	IL36RN (IL-36 receptor antagonist)	Fever, neutrophilia, pustular psoriasis	Acitretin, corticosteroids, TNF inhibitors, methotrexate, cyclosporine, phototherapy
Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome	lipodystrophy and elevated temperature (CANDLE) syndrome PSMA3, PSMB4, PSMB8, PSMB9 (proteasome subunits)	Fever, progressive facial lipodystrophy, periorbital edema	JAK inhibition with baricitinib, methotrexate, corticosteroids, cyclosporine, azathioprine, IVIG
STING-associated vasculopathy with onset in infancy (SAVI)	TMEM173 (STING)	Acral vasculitis increasing with cold, pustular lesions	Baricitinib, tofacitinib, ruxolitinib, corticosteroid

IL: Interleukin, IVIG: Intravenous immunoglobulin, JAK: Janus Kinase NSAIDs: Nonsteroidal anti-inflammatory drug; STING: Stimulator of interferon gene; TNF: Tumor necrosis factor.

Table 2. Multifactorial autoinflammatory diseases [10].

Multifactorial autoinflammatory C	Genes	Clinic Manifestation	Treatment
Hidradenitis suppurativa (HS)	Unknown	Nodules with ulceration, abscesses, and fistulas; evolve into hypertrophic scars	Adalimumab, infliximab, ustekinumab, anakinra, antibiotics, corticosteroids
Generalized pustular psoriasis (GPP)	CARD14, IL36RN	Widespread subcorneal pustules overlying erythematous plaques	Retinoids, cyclosporine, methotrexate, infliximab, gevokizumab, canakinumab, IL-17A inhibitors
Palmoplantar pustular psoriasis (PPPP)	CARD14	Sterile pustules on palms and soles; hyperkeratosis and fissuring	PUVA, UVB, acitretin, methotrexate, corticosteroids, cyclosporine, ustekinumab
Synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO)	PSTPIP2, LPIN2, NOD2, IL1RN, unknown	Osteomyelitis, hyperostosis, synovitis, acne, fissure	NSAIDs, methotrexate, sulfasalazine, bisphosphonates, TNF inhibitors, ustekinumab, secukinumab, anakinra
Pyoderma gangrenosum, acne, and suppurative hidradenitis (PASH)	PSTPIP1, NLRP3, MEFV, NOD2, PSMB8, NCSTN	Suppurative hidradenitis; acne; pyoderma gangrenosum later	Anakinra, infliximab, adalimumab
Behcet's disease (BD)	MEFV, HLA-B51, TNFAIP3, complex	Oral aphthous ulcers; genital ulcers; erythema nodosum, vasculitis	Colchicine, corticosteroids, azathioprine, thalidomide, cyclosporine, anakinra, cyclophosphamide, TNF inhibitors, canakinumab, tocilizumab
Systemic juvenile idiopathic arthritis (SJA)	Complex	Maculopapular rash, arthritis, fever, serositis, hepatosplenomegaly	Corticosteroids, NSAIDs, canakinumab, anakinra, tocilizumab
Adult-onset Still's disease (AOSD)	Complex	Maculopapular rash, arthritis, fever, serositis, hepatosplenomegaly	Corticosteroids, DMARDs, TNF inhibitors, anakinra, canakinumab, tocilizumab
Schnitzler's syndrome	Unknown	Urticarial-like lesions with neutrophilic infiltrates, fever, arthritis, IgM gammopathy	Corticosteroids, anakinra, canakinumab, rilonacept
Sweet's syndrome (acute febrile neutrophilic dermatosis)	HLA-B54, PTPN6, IDH1, MEFV, unknown	Erythematous papules, nodules, and plaques, fever, leukocytosis	Corticosteroids, colchicine, dapsone, potassium iodide, anakinra, TNF inhibitors
Pyoderma gangrenosum (PG)	MEFV, NLRP3, NLRP12, NOD2, LPIN2, PSTPIP1, JAK2, MTHFR, complex.	Sterile pustules evolve into ulcers with undermined borders	Corticosteroids, antibiotics, IVIG, thalidomide, infliximab, ustekinumab, canakinumab, anakinra
Psoriasis	CARD14, IL36RN, TNFAIP3, TNIP1, unknown	Papules and plaques with silver scale, typically on extensor surfaces; sterile pustules; hyperkeratosis	Corticosteroids, retinoids, phototherapy, methotrexate, cyclosporine, TNF inhibitors, ustekinumab, secukinumab, ixekizumab, apremilast
Acne vulgaris	Complex	Comedones; papules; pustules; nodules; cysts	Antibiotics, retinoids, salicylic acid, spironolactone, nitric oxide-releasing .

DMARD: disease-modifying antirheumatic drug;IL: interleukin; IVIG: intravenous immunoglobulin; NSAIDs: nonsteroidal anti-inflammatory drug; PUVA: psoralen and ultraviolet- A; Anti-TNF:tumor necrosis factor inhibitor drug;UVB: ultraviolet B; IgM: immunoglobulin M gamopathy.

bowel disease, heart disease, metabolic syndrome and malignancy. In mouse studies with malignant melanomas, IL-18 inhibition has been shown to reduce the development of vascular cell adhesion molecule-1 (VCAM-1), reducing the development of metastasis [8]. Thus, IL-18 inhibition strategies are targeted in both inflammatory diseases and cancer treatment.

Proteasome immunoproteasome

In some patient groups, the absence of clinical response with IL-1 inhibitory therapy and continued studies investigating the pathogenesis of the disease led to the identification of proteasome-immunoproteasome components. Proteasome-immunoproteasomes are several multiprotein structures that are responsible for the removal of intracellular and foreign cell waste. After recognition of the Type-1 IFN receptor by the cell surface, Janus Kinase (JAK) and the transducer and activator transcription factor (STAT) are stimulated. These all together cause increased production of IFN, formation of cell damage associated oxygen radicals and nitrogen proteins. These proteins are cleared from the cell by proteasomes and immune proteasomes in sake of cell survival [9].

Autoinflammatory diseases are divided into two either as monogenic autoinflammatory diseases or multifactorial autoinflammatory diseases, according to the detected genetic mutations. In monogenic diseases, a single gene region has been associated with the disease while in multifactorial diseases, many gene mutations have been associated with the disease. Table-1 presents monogenic autoinflammatory diseases and associated gene mutations and Table-2 presents multifactorial autoinflammatory diseases and related gene mutations [10].

Behcet's disease

Behcet's disease (BD) is included in the multifactorial autoinflammatory diseases group. Common clinical signs of autoinflammatory diseases include; oral aphthae, arthritis, papulopustular skin lesions, pathergy test positivity, uveitis, meningoencephalitis, genital aphthae, epididymoorchitis, lymphadenopathy and amyloidosis. Since the clinical findings of BH are similar to the clinical findings of autoinflammatory diseases, studies have been conducted on this subject, considering that BH could be an autoinflammatory disease. In table-3, the findings of BH that overlap with the clinic of autoinflammatory diseases are schematized [11].

Table 3. Clinical findings of Behcet's disease that overlap with autoinflammatory diseases [11].

Behcet's Disease's Clinic Finding	Autoinflammatory Diseases
Oral aphthous ulcers	MKD, TRAPS, CAPS
Skin pathergy reaction	PAPA
Arthritis	FMF, TRAPS, PAPA, Blau's Syndrome, CAPS, MKD
Papulopustular/acne like lesions	DIRA, PAPA
Meningoencephalitis	FMF, CAPS
Uveitis	CAPS, TRAPS, Blau Syndrome
Genital aphthous ulcers	MKD
Orchepididymitis	FMF
Amyloidosis	All autoinflammatory diseases

FMF: Familial Mediterranean fever; TRAPS: Tumor Necrosis Factor Receptor Associated Periodic Syndrome; CAPS: Cryopyrin-associated periodic syndrome; PAPA: Pyogenic Arthritis, Pyoderma Gangrenosum, Acne; DIRA: Deficiency of the interleukin-1-receptor antagonist; MKD: Mevalonate kinase deficiency.

Pathogenesis of Behcet's disease

The over-activated natural immune response in BD triggers the release of T helper-1 and T cell 17 (Th17). Natural immune system cells are predominated in the early stage of the pathological findings of the disease [12]. Neutrophilic vasculitis is a well-established pathological finding in BD. Changes in T cell balance are in favor of an increase in Th1 / Th17 and a decrease in T regulator cell (Treg). Increased Th17 causes elevation of IL-17, IL-23 and IFN- γ . Increased neutrophil infiltration develops as this pathway becomes active [13]. Pathologies associated with BH are schematized in Figure-3 and 4 [14].

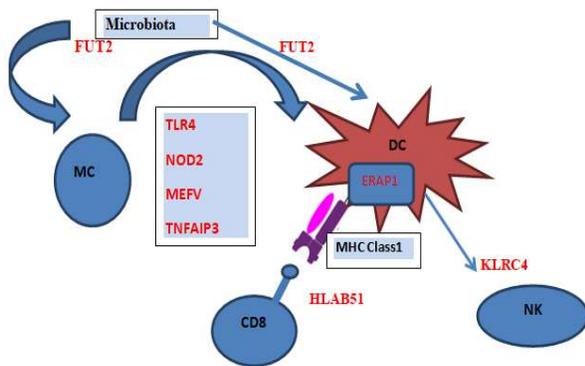


Figure 3. Immunopathology related to innate immune system in Behcet's disease (MC: Macrophages; DC: Dendritic cell; CD8: CD8 T cell; NK: Natural killer cell) [14].

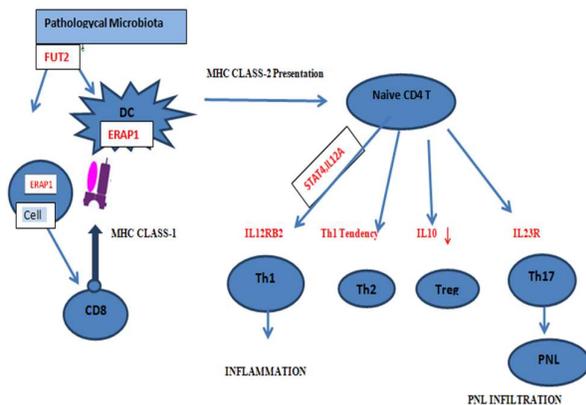


Figure 4. Immunopathology related to adaptive immune system in Behcet's Disease (DC: Dendritic cell; CD8: CD8 cytotoxic T cell; Th1:T helper1cell; Th2: Thelper2 cell; Treg: T regulatory cell; Th17:T 17 cell; PNL: neutrophils) [14].

On the other hand, there are studies showing that some microorganisms contribute to disease in susceptible individuals. Some streptococcal derivatives such as *Streptococcus sanguinis*, herpes simplex type-1 cause cross-reaction by showing homologous structure with human heat shock proteins (HSP) and have been shown to activate the immune system [15]. BH has been associated with microorganisms such as *Borrelia burgdorferi*, *Helicobacter pylori*, Cytomegalovirus, Epstein Barr virus, parvovirus, varicella zoster, but their relationship is unclear since these publications are consisted of few cases. Nevertheless, the role of microorganisms in disease pathogenesis is thought to be in the form of exacerbation of the disease [13,14].

Gene studies in Behcet's disease

Genetic studies conducted to elucidate Behcet's disease pathogenesis showed the presence of gene mutations associated with the natural immune system in this condition. Genetic mutation studies detected in BD are presented in Table 4 [14]. MEFV gene mutations are mutations detected in FMF patients and have also been detected in BD. However, the relationship of MEFV gene mutation with Behcet's Disease is not clear [16].

The genome region encoding IL10 is the first gene region detected in BD. Although different missense variants in this gene region differ among communities, some variants have been shown to be associated with BD [14]. These variations have been associated with the formation of autoinflammation by decreasing the release of IL-10 from macrophages and not showing the effect of IL-10 in limiting inflammation. There are studies showing that IL-10 level is lower in BD than healthy individuals [17].

Table 4. Gene studies of Behcet's disease [14].

Variant	Gene	Location	Function of the risk allele
rs1495965	IL23R,IL12RB2	Intergenic	
rs924080	IL23R,IL12RB2	Intergenic	
rs1518111	IL10	Intron	Reduces expression in monocytes
rs1800871	IL10	Promoter	
rs9494885	TNFAIP3	Intergenic	No difference in expression in PBMCs
rs7574070	STAT4	Intron	Increases expression
rs897200	STAT4	Intergenic	Increases expression of STAT4 and IL17
rs7616215	CCR1	Intergenic	Decreases expression in monocytes, reduces monocyte chemotaxis
rs13092160	CCR1, CCR3	Intergenic	Decreases expression in PBMCs
rs2617170	KLRC4	Missense	
M694V	MEFV	Missense	Increases response to LPS
rs17482078	ERAP1	Missense	
rs681343	FUT2	Synonymous	
rs17810546	IL12A	Intergenic	
R381Q,G149R	IL23R	Missense	Reduces IL-23 dependent IL-17
D299G, T399I	TLR4	Missense	Reduces response to LPS, hyporesponsiveness to endotoxin
R702W, G908R L1007fs	NOD2	Missense Frame shift	Reduces response to MDP

PBMC: Peripheral blood mononuclear cell; LPS: lipopolysaccharide; MDP: muramyl dipeptide.

Li et al. detected TNFAIP3 mutation in patients with 722 BH [18]. TNFAIP3 is the region encoding the ubiquitin modified enzyme A20. It plays an important regulatory role in the NF- κ B signaling pathway and provides TNF, toll like receptors (TLRs), IL-1R and NOD2 release.

The p.Arg725Gln, variant gene in the ERAP1 gene has been studied and detected in the

Turkish population, and found to be higher in patients with positive HLA B51 allele [19]. The fact that the disease is seen more in some geographical regions suggested that this variant may be due to mutations. ERAP1 encodes aminopeptidase-1 in the endoplasmic reticulum and contributes to the production of the N-terminal peptide suitable for antigen binding for the MHCclass-1 of the proteasome.

Missense variant gene rs2617170 was detected in KLRC4 in the Turkish and Japanese population. This variation is related to the natural killer (NK) cell gene complex region. This variation was found in 23 of 83 BD patients. It is thought to cause autoinflammation by causing a communication-related pathology between the NK cell and the MHC gene [20]. However, the effect of these variations is still unclear.

The intergenic region between IL23R-IL12RB2 detected in BD is thought to cause increased expression of IL23R. IL23R is expressed in TH17 and macrophages [21].

Intergenic and intron gene regions have been detected in STAT4-related gene regions. STAT4 causes IL12 and IL23 secretion and Naive T cell to transform into Th1 and Th17 [22].

Genetic studies associated with FUT2 have suggested that it may be associated with disease exacerbation by affecting intestinal bacterial flora in BD [23].

On the other hand, Behcet's Disease has a strong relationship with HLA-B51, and its degree of relationship has been found at different rates in different populations [24]. Some variant genes have also been shown to be associated with BD, albeit weak, at the HLA-A locus [25,26]. Ombrello et al. detected 16 variants of HLA B mature protein amino acid sequence in BD patients by performing HLA Class 1 gene analysis with the Genome wide association studies (GWAS) method. This variation has been shown to cover antigen-binding protein regions in MHC Class-1. It includes the antigen-binding protein region in variations detected at the HLA-A locus. It has been supported that these variations may lead to a pathology in the MHC Class 1 binding site, causing a problem in cytotoxic T cell or natural killer cell (NK) communication and causing

inflammation [27]. In another study, the variation in amino acid sequence has shown that the communication between HLA-B and NKIR and KIR3DL1 / KIR3DS1 associated with cytotoxic T cell regulation is affected [28]. Also, ERAP1 affects cytotoxic T cell and NK cell communication with MHC Class1. Gene defects associated with ERAP1 were also detected in Behcet's disease. Recent studies have focused on ERAP1 [29,30]. With all these results, the disease is thought to occur due to defect in MHC Class-1 and NK cell and cytotoxic T cell communication

Conclusion

Gene studies, pathological data, and cytokines related to Behcet's disease pathogenesis show that the disease may have developed due to defects in multiple immune pathways. However, the natural immune system is an important step. With GWAS gene studies, it is thought that more progress will be made on the etiopathogenesis of the disease.

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ORCID iD of the author(s)

Sengul Aksakal / 0000-0001-7818-5680

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