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ADENOSINE DEAMINASE ACTIVITY IN PATIENTS WITH ANKYLOSING SPONDYLITIS*
ANKİLOZAN SPONDİLİT HASTALARINDA ADENOZİN DEAMİNAZ AKTİVİTESİ

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ABSTRACT

Although the pathogenesis of ankylosing spondylitis (AS), which is a systemic disease characterized by inflammation, is largely unknown, pro-inflammatory mediators, oxidative stress and immunity are thought to be involved in the development and the prognosis of the disease. It was aimed in this study to reveal activities of adenosine deaminase (ADA), a cornerstone enzyme in different pathways. Twenty nine AS patients and 16 healthy volunteers were included in the study. Patients were divided into two groups as active and inactive phases. Additionally, the patients were re-grouped according to axial/peripheral involvement. ADA and myeloperoxidase (MPO) activities, and advanced oxidation protein products (AOPP) levels were measured in plasma of the study groups. While the significant increases were observed in AOPP and MPO levels in AS patients compared to the control group, when the patients were divided into subgroups, only ADA was significantly decreased in active patients. On the other hand, there was no significant difference in AOPP, MPO and ADA levels in groups created according to axial/peripheral involvement. Based on these findings, it is thought that the decrease in ADA levels in AS patients can give an idea about the prognosis of the disease and can be used as an activity marker.

Keywords: Adenosine deaminase, advanced oxidation protein products, ankylosing spondylitis, autoinflammation, myeloperoxidase.

ÖZ

Enflamasyon ile karakterize sistemik bir hastalık olan ankilozan spondilit (AS)'in patogenezi büyük ölçüde bilinmemekle birlikte proenflamatuvar mediyatörler, oksidatif stres ve immünitenin hastalığın gelişiminde ve prognozunda rol oynadığı düşünülmektedir. Bu çalışmada AS hastalarında, farklı yolaklarda köşe taşı bir enzim olan adenozin deaminaz (ADA)'ın aktivitesinin gösterilmesi amaçlanmıştır. Çalışmaya 29 AS hastası ve 16 sağlıklı gönüllü dâhil edildi. Hastalar aktif ve inaktif faz olarak iki gruba ayrıldı. Ek olarak hastalar aksiyel/periferik tutuluma göre yeniden gruplandırıldı. Çalışma gruplarının plazmalarında ADA ve miyeloperoksidaz (MPO) aktiviteleri ve ileri oksidasyon protein ürünleri (Advanced Oxidation Protein Products; AOPP) seviyeleri ölçüldü. AS hastalarında AOPP ve MPO düzeylerinde kontrol grubuna göre anlamlı artışlar görülürken, hastalar alt gruplara ayrıldığında sadece aktif hasta grubunda ADA aktivitesinde anlamlı azalma görüldü. Diğer taraftan, aksiyel/periferal tutuluma göre oluşturulan gruplarda AOPP, MPO ve ADA düzeylerinde anlamlı farklılık yoktu. Bu bulgulara dayanarak, AS hastalarında ADA düzeylerindeki düşüşün hastalığın prognozu hakkında fikir verebileceği ve aktivite belirteci olarak kullanılabileceği düşünülebilir.

Anahtar kelimeler: Adenozin deaminaz, ankilozan spondilit, ileri oksidasyon protein ürünleri, miyeloperoksidaz, otoenflamasyon

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INTRODUCTION

Ankylosing spondylitis (AS), a major subgroup of rheumatoid diseases called spondyloarthritis, is a common chronic inflammatory disease that affects the axial skeleton, causing structural and functional disorders and decreased quality of life. Its prevalence in the general population ranges from 0.15 to 0.80% (1, 2).Like the other forms of spondyloarthritis, the etiology of AS is not fully known (2). However, the relationship of inflammatory agents such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β, IL-17, and IL-23, which are involved in the pathogenesis of AS, has revealed that increased inflammation and oxidative stress also play important roles in the pathogenesis of the disease (2-4). Additionally, similar to other systemic autoimmune diseases, the imbalance between innate and adaptive immunity has been reported to participate in progression (5).

Adenosine deaminase (ADA) is an immune regulatory molecule shown to have the clinical value in autoimmune diseases (5). ADA, an enzyme catalyzing the deamination of adenosine, which is formed by the breakdown of ATP in low energy charge and increased cellular stress, acts as an immunosuppressive signal and prevents excessive inflammation (6, 7). Decreased ADA activity leads to the abnormal adenosine concentration, which influence the immunity. Moreover, it has been shown that ADA activity changes in autoimmune diseases and could be used as a biomarker. Total ADA activity is the sum of isoenzymes (ADA1 and ADA2); ADA1 is an intracellular protein while ADA2 is a plasma protein (5).

Although the use of ADA activity in different purposes such as diagnosis, disease activity, and prognosis in autoimmune diseases like rheumatoid arthritis (RA) and SLE has been shown in the literature, similar studies in AS patients are quite limited and the results obtained have been conflicting (8, 9).

The current study has shown the possible relationship between ADA activity and disease activity, and axial/peripheral involvement in AS patients. Thus, it will be possible to put forward as a new marker candidate, especially by showing the contribution of ADA to the pathogenesis and activity prediction of the disease. Also, oxidative stress is closely associated with inflammation status, and the maintenance of oxidant/antioxidant status is known to modulate immune system homeostasis the changes in myeloperoxidase (MPO) and advanced oxidation protein products (AOPP) levels were also examined (10).

MATERIALS AND METHODS

This study was conducted in Departments of Physical Medicine and Rehabilitation, and Clinical Biochemistry. Prior to the study, approved by the Local Ethics Committee (No: 2019/804), informed consent was obtained from all patients.

Study Groups

Twenty-nine AS patients (F/M: 11/18) who met the modified New York diagnostic criteria (11), and 16 healthy volunteers (F/M: 8/8) were included in the study. The inclusion criteria for patients were diagnosis of AS; being on disease-modifying anti-rheumatic drug (DMARD) for at least three months, and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) for at least four weeks.

Patients with uncontrolled hypertension, decompensated diabetes mellitus, history of coronary artery disease, fibromyalgia, and acute inflammation were excluded.

The pain levels of patients with AS were evaluated with a 10-cm visual analog scale (VAS), while their functional status was evaluated with the "Bath AS Functional Index" (BASFI)(12, 13).

The disease activity was evaluated with the Bath AS Disease Activity Index (BASDAI), and the patients were divided into two groups as those in the active and inactive phase (14). According to their BASDAI levels, patients with BASDAI≥ 4 were considered active (15).

In addition, the groups were re-formed according to axial and peripheral involvement.

As the control group, volunteers selected among healthy individuals who had not used any medication for the past month, including vitamin and/or mineral preparations; have routine laboratory results are within reference limits, without any systemic disease, similar to the age and gender distribution of AS patients, were included in the study.

Biochemical Analyses

C reactive protein (CRP) and erythrocyte sedimentation rate(ESR) levels of AS patients were measured using appropriate commercial kits in Roche Cobas c702 and Vision ESR Analyzer, respectively.

Blood samples were centrifuged at 1500 g for 15 minutes at +4°C, and the plasma obtained were stored at -80°C until the day of study to be used in measuring ADA and MPO activities, and AOPP levels.

Plasma MPO activity was determined by the spectrophotometric method of Bradley et al. (16).It was based on kinetic measurement at 460 nm of the formation rate of the colourful product of the oxidation of o-dianisidine with MPO. One unit of MPO was defined as the enzyme catalyzing the oxidation of micromol o-dianisidine per minute (U/L; μmol o-dianisidine/min/L). The CV value of the method used in determining MPO activity was found to be 3.12%.

Determination of AOPP was based on a spectrophotometric assay according to Witko-Sarsat et al. (17). AOPP levels were expressed in micromoles of chloramine-T equivalents per litre of plasma (μmol/L). The CV of the method used in the AOPP measurement was found to be 3.42%.

The ADA activity was measured according to the method of Giusti and Galanti(18), that is, the formation of colored indophenol complexes and quantitated spectrophotometrically. One unit of ADA is defined as the enzyme required to synthesizeone micromole of ammonia from adenosine, in one minute. The CV of the method was measured as 4.80%.

Statistical Analysis

Descriptive analyses were performed on the data available to all individuals randomly divided as control group, AS group, and sub-groups. Shapiro-Wilk test was used to test whether data was normally distributed. Continuous variables with normal and abnormal distributions were expressed as mean with standard deviation (SD) and median [interquartile range (IQR)], respectively. Categorical variables were reported as per-

centages. While comparing quantitative data between groups, Student's t test was used for those which conformed to the normal distribution, and Mann-Whitney U test was used for those which did not conform to the normal distribution. Spearman's correlation coefficients were calculated to study the relations between ADA and BASDAI scores. ANOVA or Kruskal Wallis tests were used for comparing subgroups and control; the significance between the two groups was determined by the Tukey's multiple comparison test and Mann–Whitney U test with Bonferroni correction respectively. Chi-square test was used to compare qualitative data. p<0.05 were considered statistically significant.

RESULTS

According to Table I, no significant difference was observed between the subgroups in terms of age and gender. However, when the subgroups were compared, there was a significant difference between the active

and inactive groups only in terms of the types of drugs they used.

As a result of the comparison of total patients with the control group, there was no significant difference in age, gender, and ADA levels, while MPO and AOPP levels were found to be increased (Table II).

As shown in Table III, CRP, ESR, VAS and BASFI levels were found to be higher in active patients according to inactive group, but no difference was observed when patients with axial and peripheral involvement were compared.

Also, as seen in Figure I, a significant correlation was shown between ADA and BASDAI in AS patients (p< 0.001; r= - 0.622).

DISCUSSION AND CONCLUSION

The adenosine pathway is a mechanism to modulate inflammatory response and prevent tissue damage. In this context, the degree of ADA concentrations and/or

Table I. Demographical data of study groups

		Sub-Groups			Sub-Groups		
	Control (n=16)	Active (n=13)	Inactive (n=16)	p	Axial (n=18)	Peripheral (n=11)	p*
Age (Years)	36.31±4.80	33.15±6.94	34.06±6.42	0.718	34.89±5.76	31.64±7.53	0.200
Gender (F/M)	8 (50%)/ 8 (50%)	7 (53.8%)/ 6(46.2%)	4(25%)/ 12(75%)	0.143	6(33.3%)/ 12(66.7%)	5(45.5%)/ 6(54.5%)	0.696
Duration of Disease (Years)	-	4.00 (6.50)	6.00 (9.75)	0.824	6.00 (10.13)	3.00 (5.0)	0.149
Type of Medication (DMARD/NSAID)	-	5(38.5%)/ 8(61.5%)	15(93.8%)/ 1(6.3%)	0.003	13(72.2%)/ 5(27.8%)	7(%63.6)/ 4(%36.4)	0.694

p = Differences between active and inactive groups; p*, differences between axial and peripheral involvement. The data were expressed as mean± SD; mean± standard deviation or median (IQR); median (interquartile range) ; NSAID, Non-Steroidal Anti-İnflammatory Drug ; DMARD, Disease Modifying Anti-Rheumatic Drugs

Table II. Comparison of demographic and biochemical parameters of AS patients and control groups

	GROUPS		
	AS (n=29)	Control (n=16)	p
Age (Years)	33.65±6.55	36.31±4.80	0.162
Gender (F/M)	11(37.9%)/18(62.1%)	8(50%)/8(50%)	0.433
MPO (U/L)	86.78±24.52	69.07±12.98	0.003
AOPP (µmol/L)	148.28±32.77	61.61±27.78	0.005
ADA (U/L)	15.41±2.31	15.16±2.58	0.738

p = Statistically significant differences between AS and control patients; The continous data was expressed as mean± SD.

Table III. Comparison of AS patients' subgroups and control group

		Subgroups			Subgroups		
	Control (n=16)	Active (n=13)	Inactive (n=16)	p	Axial (n=18)	Peripheral (n=11)	p*
ESR(mm/hr)	2.5(1.75)	14.0 (16.45)[a]	4.0 (1.75)[a]	<0.001	4 (6.00)[a]	3 (1.80)[a]	0.39
CRP(mg/L)	0.9 (1.42)	13.0 (10.00)[a]	3.40 (0.30)[a]	<0.001	6.25 (9.95)[a]	3.40 (9.50)[a]	0.284
BASDAI	-	5.83±1.57	1.97±1.24	<0.001	3.45±2.00	4.11±2.79	0.523
BASFI	-	4.06±2.62	1.76±1.33	0.010	2.90±1.15	2.62±1.60	0.774
VAS(cm)	-	5.31±3.05	2.74±2.12	0.013	4.11±2.37	5.73±2.57	0.047
AOPP (µmol/L)	61.61±27.78	139.33±15.43[a]	150.55±29.97[a]	0.313	138.04±15.93[a]	157.76±32.01[a]	0.219
MPO (U/L)	67.82±11.84	96.06±14.20[a]	86.11±14.17[a]	0.127	87.26±15.11	95.98±13.19[a]	0.308
ADA(U/L)	15.16±2.58	13.87±1.82	16.66±1.88	<0.001	15.60±2.32	15.10±2.74	0.579

[a], statistically significant differences between control and other groups; p, differences between active and inactive groups; p*, differences between axial and peripheral involvement. The data were expressed as mean±SD; standard deviation or median (IQR) interquartile range.
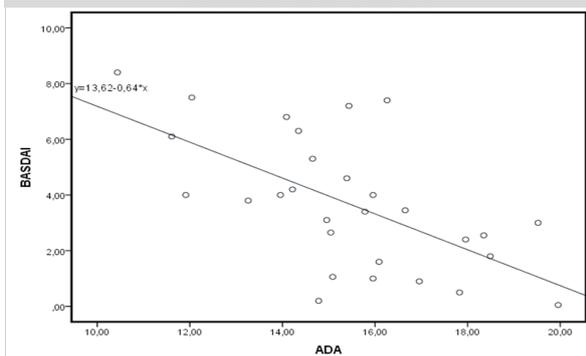
Figure I. Correlation between ADA activities and BASDAI levels in the AS patients

activity involved in the regulation of extracellular adenosine concentrations by catalyzing the irreversible conversion of adenosine to inosine plays an important role in the modulation of purinergic responses to various pathophysiological events such as chronic lung diseases (19), RA(20, 21), inflammatory bowel diseases and sepsis (22, 23). It has also been shown that determination of serum ADA activity will be used as a determinant of disease activity in RA patients (24). However, up to date, the number of articles investigating ADA levels in AS patients has so far been very limited.

Similar to the present study, in previous studies, there was no difference between the AS patients and control groups in terms of ADA levels (8, 9,25). However, Zhaowei et al., have reported that ADA2 levels were significantly higher in the patient group (8). However, the results seem contradictory when it is considered that serum ADA activity is largely due to the ADA2 isoenzyme (5). This may be due to the differences used in the experimental method.

The principles and aim of AS management is to control disease activity, achieve remission, and improve the quality of the life of patients, evaluation of disease activity, especially in the early period, is seems important (26, 27). In the current study, ADA levels, which unchanged compared to the control group, were lower in the active group. In addition, in the current study, the strong correlation between BASDAI levels and ADA levels in the patient group supports this finding. This decrease in ADA activity probably causes an increase in adenosine levels. This can be considered as a compensation mechanism for the organism to suppress excessive immune and inflammatory response. Further studies on this subject are needed.

ADA, due to adenosine, can also modulate reactive oxygen species (ROS) generation in neutrophils based on the receptor subtype activated (6, 22, 26). In most inflammatory arthritides, neutrophils are the most abundant cells in synovial fluids (6). Neutrophils and macrophages are known to cause direct damage to bone and cartilage tissue in the joints with the formation of ROS, thus playing an important role in the pathogenesis of chronic inflammatory joint diseases that progress with tissue damage (28-31). Oxidation-modified albumin derivatives secondary to increased ROS formation, such as AOPP have been shown to be increased in many pathologies associated with oxidative stress and have been recognized as a stable marker reflecting the extent of

oxidative stress and inflammation, and oxidative protein damage (32).The increase in AOPP levels, which are considered to be the product of MPO, allows the evaluation of oxidative stress and inflammation together (17, 32).

In the present study, when total patients compared to healthy controls, a significant increase was shown in MPO and AOPP levels, which indicate oxidative stress and inflammation, compared to healthy controls. These results seem to be consistent with similar studies in the literature that indicate generally increased levels of oxidative stress in these patients (29-31,33). Also, there is also a study showing that MPO values do not change (9).

However, in the present study, MPO and AOPP levels, which were found to be high in AS patients, were not affected by the activity status of the disease.Yazici et al. (29)reported that higher MPO and AOPP levels in active patients. The reason for this difference may be that Yazici et al. (29) have evaluated BASDAI and CRP levels together in the selection of active patients and used different cut-off values.

In this study, we also compared patients with axial/ peripheral involvement as different subgroups. However, there was no difference in terms of AOPP, MPO and ADA levels in the comparisons which made according to the involvement of the disease. In contrast, Yazici et al. (29) showed that MPO and AOPP levels increased in the group with peripheral involvement. However, the activity status of the disease, which they showed to affect both values, was not evaluated in the axial / peripheral involvement subgroup. In the present study, there was no statistical difference between the number of active/ inactive patients in both the treatment subgroup and the involvement subgroup (data not shown).

As a result, in this study, although the fact that ADA levels did not change in AS patients compared to the control group suggests that it does not contribute to the pathogenesis, it has been demonstrated that it is appropriate to use the decrease in ADA levels as an activity indicator.

Conflict of Interest
The authors declare no conflict of interest.

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