RESEARCH ARTICLE

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Tear Ozmolarity in the Evaluation of Ankylosing Spondylitis Disease Activity

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Objective: To investigate the frequency of dry eye syndrome (DES) in patients with ankylosing spondylitis (AS) by measuring tear osmolarity (TO) with the recently introduced TearLab system (TearLab Corp, San Diego, Calif.) and to determine the relationship between the severity of DE and AS activity.

Materials and Methods: Fifty-seven eyes of 57 patients and 29 healthy individuals as the control group were included. TO measurements, tear break-up time (BUT), and Schirmer's tests were performed. The patients were divided into three main groups based on their Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores: healthy controls, mild-moderate (BASDAI ≤ 4), and severe (BASDAI>4) , into another group based on HLA B27 status: control, HLA B27 (+) and (-), finally based on treatment status: control, treatment (+) and (-).

Results: There were no significant differences based on BASDAI among these subgroups in terms of TO (P= 0.383), BUT (P= 0.722) and Schirmer's scores (P= 0.108). Also disease activity score values were not correlated with TO values (r=0,23, P=0,863), Schirmer's scores (r= –0.223, P= 0.095) and BUT scores (r = –0.044, P= 0.744).

Conclusion: In our study, we determined higher TO values in non-treated, HLA-B27 marker positive and high disease activity groups. Although these values did not reach statistical significance, our findings suggest that in high inflammatory levels, TO can be utilized as a preliminary marker for AS disease activity.

Keywords: *Ankylosing spondylitis, dry eye, schirmer's test, tear break-up time, tearlab, tear osmolarity*

Ankilozan Spondilit Hastalık Aktivitesi Değerlendirmesinde Gözyaşı Ozmolaritesi

Amaç: Ankilozan spondilitli (AS) hastalarda TearLab sistemi (TearLab Corp, San Diego, Calif.) ile gözyaşı ozmolaritesini (TO) ölçerek kuru göz sendromunu (DES) değerlendirmek ve göz kuruluğu ile AS arasındaki ilişkiyi saptamak.

Gereç ve Yöntemler: Çalışmaya 57 hastanın 57 gözü ve kontrol gurubu olarak 29 sağlıklı göz dahil edildi. Çalışmaya alınan hastalar, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) skorlarına göre; kontrol, hafif-orta (BASDAI ≤ 4), ve şiddetli (BASDAI>4) olarak; HLA B27'ye göre; kontrol, HLA B27 (+) ve HLA B27 (-) ve tedavi statüsüne göre kontrol, tedavi alan (+) ve tedavi almayan (-) olarak üç ana guruba ayrıldı. Bu üç ana gurubun her biri; TO, BUT ve Schirmer testleri bakımından karşılaştırıldı.

Bulgular: Bu üç ana grup'un her biri için yapılan karşılaştırmalarda; TO (p = 0.383), Schirmer testi (p = 0.108) ve BUT (p = 0.722) değerleri açısından anlamlı bir fark gözlenmedi. Ayrıca hastalık aktivite skor değerleri ile TO değerleri (r=0,23, p=0,863), Schirmer skorları (r= –0.223, P= 0.095) ve BUT skorları (r = –0.044, P= 0.744) korele değildi.

Sonuç: Çalışmamızda; hastalık aktivitesi yüksek, HLA-B27 belirteci pozitif ve tedavi almayan hasta gruplarında elde edilen TO değerlerini daha yüksek olarak saptadık. Bu değerler her ne kadar istatistiksel anlamlılık düzeyine ulaşmamış olsa da, bulgularımıza gore yüksek inflamasyon düzeylerinde TO seviyesinin, AS hastalık aktivitesi için preliminer bir gösterge olarak kullanılabileceği kanaatindeyiz.

Anahtar Kelimeler: *Ankilozan spondilit, tearlab, gözyaşı ozmolaritesi, kuru göz, schirmer testi, gözyaşı kırılma zamanı*

Introduction

Ankylosing spondylitis (AS) is an immune-related rheumatic disease characterized by chronic inflammation and involving various organs (1, 2). Instability occurs on the corneal surface in AS with the accumulation on the ocular surface of cytokines released by T cells and with the conversion of corneal Langerhans cells (LC), which play an important role in immune processes on the ocular surface, into an active participant in corneal immune response (3-5). This inflammation has been shown not only to cause disturbance on the ocular corneal surface, but also to cause dry eye syndrome (DES) by leading to dysfunction in the lachrymal gland under the effect of the cytokines (6, 7).

Secondary Sjögren's syndrome (SS) has been shown to develop in 10% of patients with AS, and LC in primary and secondary SS have been shown to be associated with DES (8, 9). DES thus occurs in inflammatory diseases as a result of involvement of the cornea together with increased tear osmolarity.

DES is a multifactorial disease that causes injury to the ocular surface compromises tear film stability and can reduce ocular comfort (10). Various tests are used for the diagnosis of DES, including Schirmer's test, tear break-up time (BUT) and vital staining methods (fluorescein and Rose Bengal). However, none of these alone is sufficient to diagnose DES and assess its severity (10, 11). Studies have also evaluated LC morphology using confocal microscopy for the diagnosis of DES (12).

TO has recently been used both to diagnose DES and as a quantitative and objective test for determining its severity (10,11,13). However, TO has not to date been used as an objective test for showing the presence and severity of DES in AS.

The purpose of this study was therefore to investigate the presence and prevalence of DE in AS with low inflammatory activity and to reveal the association between the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and severity of DES by measurement of TO.

Materials and Methods

This study was performed at Recep Tayyip Erdoğan University Medical Faculty Physical Medicine and Rehabilitation and Eye Diseases clinics, Turkey. The study protocol followed the guidelines of the Declaration of Helsinki. Recep Tayyip Erdogan University Clinical Researches Ethics Committee approval (07.10.2016 and 2016-65) and signed informed consent forms from patients were obtained. Fifty-seven eyes of 57 patients with AS and 29 healthy person(control) were included in the study. During diagnosis of DES all patients with AS exhibited a low level of inflammatory activity (CRP <0.5 mg/dL). Patients' clinical characteristics are shown in Table 1. AS was diagnosed using modified New York criteria in the Physiotherapy and Rehabilitation Clinic (14). Seventeen patients were receiving anti-TNF alpha therapy. BASDAI was calculated during ocular examinations. BASDAI contains six questions showing disease activity and is the most widely used method of evaluating disease activity independently of inflammation status (15). Patients with SS, other immune diseases, endocrine diseases such as thyroid disorder and diabetes, ocular and systemic infections, or any ocular disease other than DES, or using ocular or systemic drugs were excluded from the study.

All tests were performed by the same ophthalmologist. All patients underwent a 5-min Schirmer's test without anesthetic, a BUT test and a routine ocular examination. The BUT test was administered using a cobalt blue light source slit lamp following fluorescein installation. The mean value from two consecutive measurements was used for analysis. Tests were performed 30 min apart. No examination was performed that might cause reflex tear secretion before measurements. The tests for the study were performed on the right eye in all cases. TO was measured for each selected eye 30 min after these tests using the TearLab Osmolarity system (TearLab Corp, San Diego, Calif.). Values exceeding 305 mOsm/L were regarded as DES (11).

Table 1. Patients' clinical characteristics

	Mean	Standard Deviation	Minimum	Maximum
Mean tear osmolarity	293.09	10.80	274.00	321.00
Mean disease activity score	4.95	1.70	1.00	8,10
Mean Schirmer's test	16.46	5.19	3.00	25.00
Mean tear break-up time	10.14	2.59	2.00	15.00
Age	40.35	10.21	19.00	58.00
Mean duration of disease	5.05	5.26	1.2	12.8

Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) concentration, blood chemistry, and complete blood count were requested from each patient. Serum levels of HLA-B27 were recorded as a qualitative (positive/negative) variable.

AS disease activity was evaluated using BASDAI. The score range for this test is 0-8. We divided patıents with negatıve CRP into three groups based on their BASDAI scores as follows subgroups: control, mild-moderate (BASDAI ≤ 4), and severe (BASDAI>4), also into three groups based on HLA B27 status as follows subgroups: control, HLA B27 (+) and (-), also into three groups based on treatment status as follows subgroups: control, treatment (+) and (-).

Statistical analyses were performed using version 22.0 of the SPSS software package for Windows (SPSS Inc., Chicago, Il, USA). The results were expressed as mean ±SD, median, minimum and maximum. Kolmogorov–Smirnov tests were used to determine whether variables were distributed normally. Sex was compared using the χ^2 test. The one way ANOVA test and Kruskal–Wallis test were used to compare variables among subgroups. An overall p value less than 0.05 was considered as a statistically significant result. Spearsman's correlation coefficients were calculated to evaluate relations between the Disease activity score values and TO, Schirmer's, and BUT scores.

Results

The mean age of the 57 patients with AS and 29 heathy person who were included in this study was 40.35 (range 19–58) years. Twenty-seven (47.4%) of the patients were female, and thirty (52.6%) of the patients were male (Table 1, 2). DES was identified in 7 (12.3%) of the participating patients with AS on the basis of TO test results.

Table 2. Groups' clinical characteristics

			N	N %
Sex	Male	Control	14	25.5%
		BASDAI<=4	21	38.2%
		BASDAI>4	20	36.4%
	Female	Control	15	48.4%
		BASDAI<=4	7	22.6%
		BASDAI>4	9	29.0%
Basdaı		Control	29	33.7%
		<=4	28	32.6%
		>4	29	33.7%
Treatment Status		Control	29	33.7%
		No	17	19.8%
		Yes	40	46.5%
Hla B 27 Status		Control	29	33.7%
		Negative	20	23.3%
		Positive	37	43.0%

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

The mean BASDAI value of the patients with AS was 4.91. The mild-moderate group consisted of 29 patients , the severe group of 28 and the control group of 29. No significant differences were determined between the three subgroups in terms of TO, Schirmer's, and BUT test results (P>0.05 for all) (Table 3, Figure 1, 2).

The patients were divided into three subgroups as the control, HLA B27(+) and (–), no significant differences were observed in terms of TO, Schirmer's and BUT test results (all p> 0.05), (Table 3).

The patients were divided into another three subgroups as the control, anti-TNF alpha treatment (+) and (–), no significant differences were observed in terms of TO and Schirmer's and BUT test results (all P>0.05) (Table 3).

Spearsman's correlation coefficients demonstrated that disease activity score values were not correlated with TO values (r=0,23, P=0,863),Schirmer's scores (r= –0.223, P= 0.095) and BUT scores (r = –0.044, P= 0.744), (Figure 3).

Table 3. Comparison of dry eye tests among the groups

		TEAR OSMOLARITY		SCHIRMER'S TEST		BREAK UP TİME	
		Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)
BASDAI	Control	289.97±8.55	291(274-300)	18.03±3.26	18(11-25)	10.66±2.26	11(6-15)
	≤4	292.46±10.37	291(275-313)	17.18±4.02	15(12-25)	10.39±1.91	10(5-12)
	>4	293.62±11.50	290(278-321)	15.76±6.10	15(3-25)	9.90±3.12	11(2-15)
	P	0.383[a]		0.108[c]		0.722[c]	
TREAT-MENT STATUS	Control	289.97±8.55	291(274-303)	18.03±3.86	18(11-25)	10.66±2.26	11(6-15)
	No	295.47±12.05	295(278-321)	16.59±5.49	15(10-25)	11.35±2.34	11(5-15)
	Yes	292.03±10.33	290(275-313)	16.40±5.13	15(3-25)	9.63±2.54	10(2-12)
	P	0.212[a]		0.162[c]		0.067[c]	
HLA B27 STATUS	Control	289.97±8.55	291(274-303)	18.03±3.86	18(11-25)	10.66±2.26	11(6-15)
	N	290.55±12.05	291(275-313)	17.45±4.12	15(10-25)	10.45±1.79	10(5-13)
	P	294.41±10.10	294(278-321)	15.92±5.66	15(3-25)	9.97±2.94	11(2-15)
	P	0.165[a]		0.151[c]		0.662[c]	
SEX	Male	293.00±9.50	293(277-313)	16.91±5.00	15(5-25)	10.40±2.47	11(5-15)
	Female	290.26±11.31	289(274-321)	17.13±4.54	16(3-25)	10.16±2.53	11(2-13)
	P	0.234[b]		0.548[d]		0.923[d]	

a: One-Way ANOVA Test, b:Independent-Samples T Test, c: Kruskal Wallis H Test, d: Mann-Whitney U Test.
BASDAI: Bath Ankylosing Spondylitis Disease Activity Index.
N: Negative, P: Positive, *P<0.05*: Statistical significant level.
SD: Standart Deviation

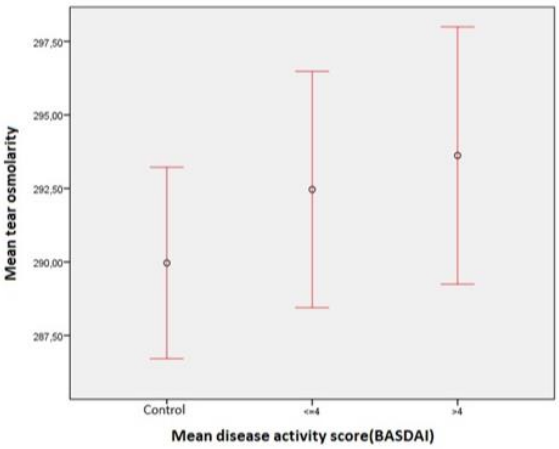

Figure 1. Comparison of tear osmolarity test among three subgroups divided base on mean disease activity score (BASDAI)

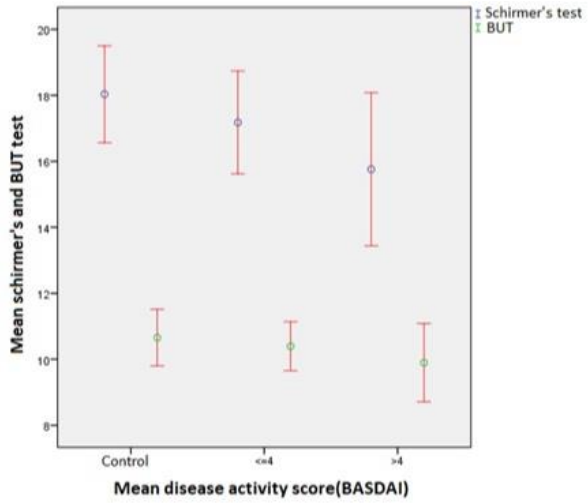

Figure 2. Comparison of Schirmer's test and Break up time (BUT) test among three subgroups divided base on mean disease activity score (BASDAI)

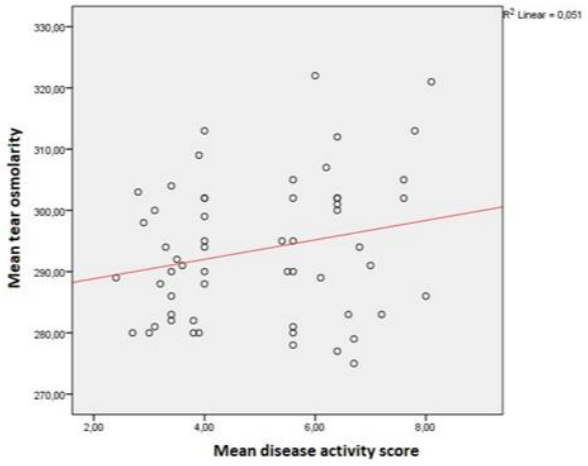

Figure 3. Correlation between mean tear osmolarity and mean disease activity (BASDAI) score in patients with ankylosing spondylitis

Discussion

In our study, there were no significant difference in terms of TO, BUT and Schirmer's test results between the groups established on the basis of BASDAI, HLA B27 and treatment status with AS.

A decrease in tear production and tear dysfunction on the ocular surface in DES, which develops as a result of various inflammatory diseases, lead to tear hyperosmolarity (16). Tear film hyperosmolarity in turn leads to osmotic stress in the surface epithelium that activates the inflammatory cascade on the ocular surface, and inflammatory mediators are released in the tear (17-19). In 2007, the International Dry Eye Workshop (DEWS) broadened the definition of DES to a multifactorial disease causing increased tear film osmolarity, ocular surface damage accompanied by inflammation, tear film instability and ocular irritability (19). TO measurement is therefore approved by many researchers as a valuable method in the diagnosis of DES, and it has for long been recommended as a potential gold standard in clinical practice (10, 20, 21).

The prevalence of DES in the normal population ranges between 5.5% and 33.7% (22). Various subjective DES tests have been used in systemic inflammatory diseases in previous studies, such as Schirmer's test, BUT, corneal and conjunctival staining, and the ocular surface disease index (23). The sensitivity and specificity of these tests are therefore lower than those of the test measuring TO using the TearLab system. In addition, these tests do not by themselves indicate severity and diagnosis. Definite diagnosis of DES in AS with secondary SS is therefore problematic. To the best of our knowledge, this is the first study to selectively evaluate TO in patients with AS. Additionally, the most important advantage of TO is its ability to reflect the diagnosis and severity of DES more objectively than other measurements (11, 20).

One study demonstrated correlations between TO findings and levels of inflammatory mediators in tears (24). The severity of DES may increase in line with the degree of increase in inflammatory activation. We therefore used TO in order to reveal the presence of DES and determine itself severity as a final objective. The TO cut-off value is important for the diagnosis of DES. Cut of values of 305 or 308 mOsm/L have generally been adopted in studies investigating the value of TO in the diagnosis of DES (10, 25). In this study we adopted a cut-off value of 305 mOsm/L. This value is sensitive in identifying patients with DES. We identified DES at a level of 12.5% according to TO measurements at low levels of inflammation. The mean age of 40.3 years and low level of inflammatory disease may account for the low level of DES observed.

Markovisky et al. (12) investigated the association between BASDAI levels as a disease activity score for AS and severity of DES using a confocal microscope and various subjective DES tests (OSDI, LIPCOF, Schirmer's, and BUT). They observed a significant

difference between low and high disease activity levels. All the patients in our study had low inflammation levels (CRP < 0,5 mg/dl), and we used the Schirmer's, BUT and TO tests as tests for DES. There was no difference among mild-moderate (BASDAI ≤4), severe (BASDAI >4) and the control groups disease activity levels in the DES tests used. However, we think that if a significant difference can be determined in terms of TO between BASDAI groups in high levels of inflammation, TO can be used as an objective test for disease activity in AS.

Markovisky et al. (12, 26) investigated the probable effects of treatment on tear production and LC in patients with AS and rheumatoid arthritis (RA). Although the patients in the group received anti-TNF alpha therapy, that study determined pathology at subjective DES tests and confocal microscopy due to high CRP levels showing systemic inflammatory status. Theoretically, the presence of DES can be explained by increased cytokines in tears in AS with SS with high systemic inflammation impairing tear production.

HLA-B27 is the gene predisposing to primary AS. In a previous study, whereas more than 90% of Caucasian AS patients was found HLA- B27(+), only about 50% of black AS patients was found positive for HLA- B27 (27). Furthermore, in another study, The prevalence of HLA-B27 in the white population was stated around 6% to 8%, with HLA- B27-05 being the predominant subtype (28). The function of these molecules is to present antigenic peptides to T cells. They play a significant role in immunological reactions at the cellular level. Laval et al. reported higher relapse, severity and complications of uveitis when they compared HLA-B27-positive patients with HLA-B27-negative (29). In other studies, however, no difference has been determined between HLA-B27-positive and HLA-B27-negative patients either in tests regarding DES or at corneal analyses using confocal microscopy, and HLA B27 has been shown to have no effect on the corneal immune mechanism (4, 26). Of the 57 patients with AS in our study, 64.9% were HLA B27-positive. No pathology was determined in either the HLA B27-positive or negative groups in terms of subjective tests as Schirmer's and BUT or in more objective dry eye test as TO test.

The main limitation of this study is that TO measurements were performed unilaterally. We performed TO measurements on the right eyes only in all patients due to the limited availability of TO kits.

Conclusions; TO, BUT and Schirmer's test results in the groups established on the basis of BASDAI, HLA B27 and treatment status were at normal levels in patients with a low level of systemic inflammation (CRP <0.5 mg/dl). Since AS is an inflammatory disease that progresses with remissions and activations, we think that objective screening of severity of DE using TO can represent a preliminary marker of AS disease activity in association with systemic inflammation level. However, since systemic inflammation levels were low in this study (CRP <0.5 mg/dL), we were unable to prove a relation between TO and disease activity (BASDAI). Further studies may now be planned to establish whether or not TO screening can be used for the definite diagnosis of DES and as a marker of AS disease activity in patients with high levels of systemic inflammation (CRP >0.5 mg/dL).

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