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Tear function alterations in patients with polycystic ovary syndrome

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Abstract. – OBJECTIVE: Complaints of dry eye are prevalent worldwide and are known to be associated with insulin resistance (IR) and hyperandrogenism. However, dry eye is often overlooked in the context of polycystic ovary syndrome (PCOS). The aim of the present study was to evaluate whether or not there is any relation between tear functions and PCOS, which is a multifaceted disorder associated with IR or hyperandrogenism.

PATIENTS AND METHODS: A total of 35 women with polycystic ovary syndrome (PCOS) were enrolled in this study, along with 27 healthy controls. Body mass index (BMI), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and free testosterone levels on the third day of menstruation were recorded, as well as hirsutism score (using the Ferriman Gallwey scoring system), insulin resistance (homeostasis model assessment), neutrophil-to- lymphocyte ratios (NLR), platelet-to-lymphocyte ratios (PLR), and mean platelet volumes (MPV). A complete ocular examination was followed by administration of the ocular surface disease index (OSDI) questionnaire and Schirmer and tear break-up time (TBUT) tests.

RESULTS: Schirmer and OSDI results were similar between groups, but TBUT was significantly lower in the PCOS group ($p = 0.002$). There were negative correlations between FG score and TBUT test ($r = -0.406$, $p = 0.001$) and between NLR and Schirmer test ($r = -0.294$, $p = 0.025$).

CONCLUSIONS: Although subjective dry eye symptoms do not present in all patients, these results confirm that tear reduction, which can cause further complications in patients with PCOS, can be detected by careful examination and sensitive tests.

Key Words:
Polycystic ovary syndrome, Insulin resistance, Hirsutism, Hyperandrogenism, Dry eye, Tear function, Neutrophil-to-lymphocyte ratio, Platelet-to-lymphocyte ratio, Mean platelet volume.

Introduction

Reduced tear secretion and complaints of dry eye are prevalent worldwide. Several studies[1-4] have demonstrated that dry eye syndrome may be related to metabolic syndrome and diabetes mellitus . These studies indicate that insulin resistance (IR), chronic hyperglycemia, oxidative stress, and hyperandrogenism can play an important role in the development of dry eye syndrome[4,5].

Polycystic ovary syndrome (PCOS) is one of the most common endocrine abnormalities, with a prevalence of 4.8-8% in women of reproductive age[6]. It is a heterogeneous endocrinopathy, causing disturbance in hormonal, reproductive, and metabolic functions. As well as being a gynecological disorder, the syndrome is associated with many metabolic and endocrine abnormalities, including IR, obesity, and predisposition to type 2diabetes[7-9]. The presence of IR is also associated with increased androgen biosynthesis and decreased levels of sex hormone-binding globulin, so playing an important role in the pathogenesis of hyperandrogenism[10,11].

Based on data from previous reports investigating the relation between dry eye and hyperinsulinemia or hyperandrogenism, this study aimed to establish whether there is any association between alterations in tear function and PCOS, which is well known to have clinical consequences that extend far beyond the reproductive system, including IR and hyperandrogenism.

Patients and Methods

Study Protocol

The study was carried out in the Faculty of Medicine at Dumlupinar University, with the ap-

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proval of the local Ethical Committee and institutional Review Board. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Written informed consents were obtained from all patients. Subjects excluded from the study included those previously diagnosed with dry eye or ocular surface disorders, and those who had intraocular surgery or nasolacrimal duct obstruction, wore contact lenses, used topical ophthalmic drugs and/or systemic medications, or had a diagnosis of idiopathic hirsutism, late onset adrenal hyperplasia, or adrenal/ovarian mass. Any patients who were on medications including oral contraceptives, ovulation induction, or insulin sensitizing agents for at least three months before the study were also excluded (Figure 1). The final sample included 62 women: 35 diagnosed with PCOS (Group 1) and 27 healthy controls (Group 2) (Figure 1). Their age, smoking status, body mass index (BMI, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and free testosterone levels on the third day of menstruation were recorded, as well as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume (MPV).

The diagnosis of PCOS was confirmed on the basis of the diagnostic criteria of the Rotterdam European Society of Human Reproduction/ American Society for Reproductive Medicine (ESHRE/ASRM 2003)[12]. Patients were eligible if they met at least two of the three major criteria: oligo/anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasonographic examination (12 or more follicles of 2-9 mm in diameter and/or ovarian volume over 10 cm^3). The Ferriman Gallwey (FG) scoring system[13] was used to measure hirsutism, which is defined by a score above 6. To determine IR, fasting glucose and serum insulin levels were measured to calculate homeostasis model assessment ratio (HOMA-R), which is calculated as follows: glucose (mg/dl) × insulin (μg/ml)/405[14].

Hormonal, Biochemical, and Blood Cell Count Analyses

After 12 hours of overnight fasting, venous blood samples were collected on the third day of menstruation into an evacuated serum separator clot activator tube (Vacuette®, Greiner Bio-One, Kremsmunster, Austria). All blood samples were centrifuged at 3500×*g* for 10 minutes at room temperature. Serum hormone concentrations were measured by the electrochemiluminescence immunoassay (ECLIA) method, using a Roche Cobas e 601 analyzer (Roche Diagnostics GmbH, Mannheim, Germany); biochemical parameters were measured using a Roche Cobas c501 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

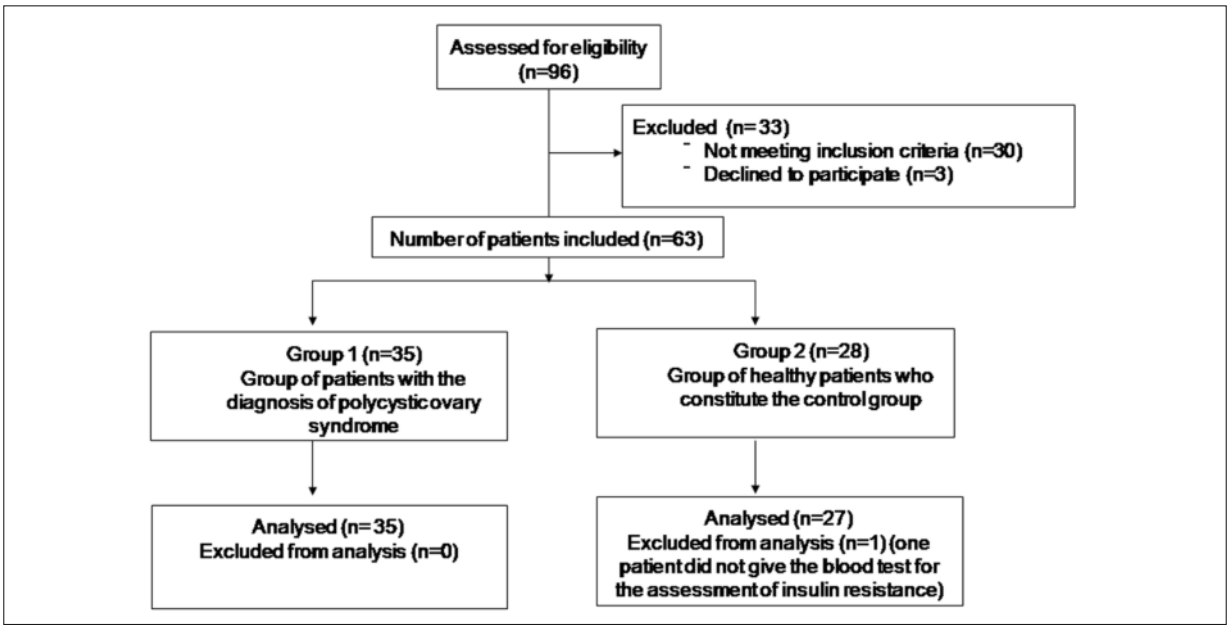

Figure 1. CONSORT flow diagram of patients enrolled in the study.

For blood cell count analysis, the blood was collected into 2.0 mL dipotassium ethylene diamine tetraacetic acid (EDTA) vacuum tubes (BD Vacuteiner® BD-Plymouth, UK). Blood cell counts were performed using Coulter Gen-S automated hematology instruments (Beckman Coulter LH 780 Gen-S System; Miami, FL, USA).

Ophthalmic Examination and Measurement

An ocular surface disease index (OSDI) validated questionnaire was administered to all subjects to assess the symptoms of dry eye before ophthalmic examination. OSDI is a standardized instrument for dry eye evaluation and can be easily performed to support diagnosis of dry eye syndrome[15]. This questionnaire has proved reliable for assessment of dry eye symptoms and has also been validated for use in Turkey[16]. After a complete ocular examination, including best-corrected visual acuity, slit-lamp, and fundus examinations, Schirmer and tear break-up time (TBUT) tests were performed.

The Schirmer I test (without anesthesia) was performed to quantify aqueous tear production. In this test, a 35x5 mm filter paper strip was used to measure the quantity of tears produced over a period of 5 minutes. Five minutes after the Schirmer I test, the TBUT test was performed, using sterile strips of fluorescein for measurement. While patients were looking upwards, the fluorescein paper was smoothly touched to the inferior fornix conjunctiva and then removed. Patients were directed to blink three times and then to look straight ahead without blinking. The tear film was observed under the cobalt-blue filtered light of a slit-lamp bio-microscope, and the interval in seconds between the last blink and the appearance of the first dry spot was accepted as the TBUT. Decreased TBUT leads to increased tear evaporation and dry eye; eyes with a Schirmer value of less than 10 mm and a TBUT of less than 10 seconds were assessed as dry eye.

Statistical Analysis

The required number of participants was determined by referring to published data from similar earlier studies. Significance value was chosen as 0.05, with beta value of 0.20 and power of 0.80. The difference of interest and the standard deviation was estimated on the basis of results from previous studies. From these results, the required sample size was calculated. SPSS for Windows version 22.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. The Kolmogorov-Smirnov test was used to analyze normality for continuous variables. Percentages were compared using the chi-square test. Student *t*-test and Mann Whitney U test were used for comparison of quantitative variables. The data were described as mean ± standard deviation, median, and percentage, according to the type of variable. Pearson correlation analysis was performed to analyze correlations between endocrine-metabolic-hematologic parameters and ophthalmic measurements; $p < 0.05$ was accepted as statistically significant.

Results

The study group comprised 35 women with PCOS, and the control group comprised 27 normal, healthy women. There was no difference between the groups in terms of clinical characteristics such as age, smoking status, BMI, day 3 FSH, LH, or LH/FSH values ($p > 0.05$; Table I). NLR, PLR and MPV values were also similar between groups ($p > 0.05$; Table I). However, free testosterone levels, FG scores, and HOMA-R values were significantly higher in Group 1 ($p = 0.004$, $p < 0.001$, and $p = 0.034$, respectively) (Table I).

Schirmer test and OSDI questionnaire results were similar between groups, but TBUT test results were significantly lower in Group 1 (9.24 ± 4.07 and 12.57 ± 5.15, $p = 0.002$). No correlation was found between Schirmer test and HOMA-R or FG scores, but there was a negative correlation between Schirmer test and NLR (r = -0.294, $p=0.025$; Table II). In addition, there was a negative correlation between TBUT and FG score (r = -0.406, $p = 0.001$; Table II).

Discussion

The impact of insulin resistance and hyperandrogenism on the eye has recently attracted considerable interest within the field of reproductive endocrinology. The effect of hyperglycemia in diabetes mellitus (DM) on the microvascular structure of the retina is well known; diabetic retinopathy, neovascular glaucoma, cataract, refractory deviation, ptosis, palsy of the oculomotorius nerve, and hordeolosis are typical ocular complications in diabetic patients[17]. Another

Table I. Comparisons of demographic, hormonal, hematologic and ophthalmologic data.

	Group 1 (n = 35)	Group 2 (n = 27)	*p*-value
Age	22.94 ± 4.43	24.93 ± 4.64	0.092
Non-smoker (%)	31 (88.6%)	22 (81.5%)	0.4
Smoker (%)	4 (11.4%)	5 (18.5%)	
BMI (kg/m^2)	2.43 ± 0.85	2.22 ± 0.80	0.260
FSH (IU/L)	4.57 ± 1.67	5.74 ± 3.17	0.066
LH (IU/L)	8.29 ± 4.76	9.37 ± 6.73	0.459
LH/FSH ratio	1.93 ± 1.06	1.92 ± 1.40	0.963
Free Test (pg/ml)	1.97 ± 1.07	1.22 ± 0.50	0.004*
FG score	7.69 ± 3.83	3.44 ± 2.22	< 0.001*
HOMA-R	3.59 ± 5.42	2.38 ± 2.10	0.034*
NLR	2.04 ± 0.87	2.28 ± 0.72	0.251
PLR	128.01 ± 39.23	121.83 ± 32.53	0.523
MPV (fL)	8.87 ± 1.09	8.62 ± 0.91	0.361
Schirmer	27.13 ± 8.09	27.72 ± 7.12	0.913
TBUT	9.24 ± 4.07	12.57 ±5.15	0.002*
OSDI	30.37 ± 16.38	32.66 ± 15.57	0.577

Abbreviations: BMI: body mass index; FSH: Follicle stimulating hormone; IU/L: International units per liter; LH: Luteinizing hormone; Free Test: Free testosterone; pg/ml: picogram per milliliter; FG Score: Ferriman Gallwey Score; HOMA-R: Homeostasis Model Assessment Ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MPV: mean platelet volume; fL: femtolitre; TBUT: tear break-up time; OSDI: ocular surface disease index. Data are presented as mean ± standard deviation for each of group. *$p < 0.05$ is considered statistically significant.

problem arising in DM is dry eye syndrome, with a reported prevalence of up to 54.3%[3]. In one of the latest studies, dry eye syndrome in type 2 DM was found to be associated with polyneuropathy, which causes corneal nerve fiber damage and reduced corneal sensitivity[1]. Other mechanisms, such as hyperglycemia resulting in advanced glycated end (AGE) product accumulation, oxidative stress and inflammation mediated by nuclear factor-kappaB (NF-κB) signaling pathways[4], quantitative and qualitative abnormalities in tear section, and poor adhesion of regenerating epithelial cells[18] were also blamed for the occurrence of dry eye in DM patients.

PCOS is a multifaceted disorder; in addition to hyperandrogenism and chronic anovulation, it is characterized by several metabolic complications that include insulin resistance and hyperinsulinemia. In the present study, the frequency of dry eye symptoms in patients with PCOS was assessed, along with any correlation with insulin resistance, hyperandrogenism, or novel hematologic parameters such as NLR, PLR, and MPV.

A combination of OSDI, TBUT, and Schirmer test has been reported as the best diagnostic test for dry eye, with sensitivity of 100%, specificity of 95%, and accuracy of 99.3%[19]. On that basis, it was decided to use those three tests in the pre-

Table II. Correlation Analysis of Schirmer test, TBUT and OSDI.

	Schirmer test		TBUT		OSDI	
	r	*p*-value	r	*p*-value	R	*p*-value
HOMA-R	-0.009	0.944	-0.125	0.333	0.134	0.299
Free Test. (pg/ml)	-0.045	0.726	-0.214	0.094	0.054	0.678
FG score	-0.078	0.544	-0.406	0.001*	0.139	0.282
NLR	-0.294	0.025*	0.056	0.678	-0.106	0.427
PLR	0.119	0.376	-0.133	0.321	-0.026	0.843
MPV (fL)	-0.237	0.073	-0.109	0.413	0.042	0.754

Abbreviations: TBUT: tear break-up time; OSDI: ocular surface disease index; HOMA-R: Homeostasis Model Assessment Ratio; Free Test: Free testosterone; pg/ml: picogram per milliliter; FG Score: Ferriman Gallwey Score; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MPV: mean platelet volume; fL: femtolitre; r: correlation coefficient; *$p < 0.05$ is considered statistically significant.

sent study; OSDI questionnaire and Schirmer test results were not statistically different, but TBUT was significantly lower in patients with PCOS. While the Schirmer test is clearly a useful screening method for diagnosing lachrymal hyposecretion, it is no more than a rough screening test, with a sensitivity that has been shown to be as low as 30-60%[20,21].

Many studies[22] have failed to demonstrate a direct relationship between different diagnostic tests. In a recent study[23] of 491 patients, no significant differences were observed between dry eye and non-dry eye groups in terms of Schirmer test value or epithelial damage, although there was a difference for TBUT value. In another study[24], tear function tests in symptomatic patients demonstrated that the results of the Schirmer I test were within the normal range (13.7 to 3.4 mm/5 minutes) in 94% of patients while TBUT was significantly reduced, which is consistent with the present findings.

In the present work, no correlation was observed between HOMA-R and any of the three ocular tests. We believe this discrepancy may be explained by the time-dependent effect of hyperinsulinemia on the eye, which should certainly be investigated in future studies.

Hyperandrogenism is another aspect of PCOS[11]. Sex hormones have been shown to impact meibomian gland functions[25]; androgens are known to affect both the lipid layer of the tear film by modulating meibomian gland secretion[26] and the aqueous layer of the tear film by modulating lacrimal gland function[27], as well as modulating the gene expressions of mucins[28]. The ourfinding of a negative correlation between TBUT test and FG score is consistent with these reports. There was also some indication of a negative correlation between TBUT and free testosterone, although this did not reach statistical significance.

Previous studies have investigated the relation between the eye and PCOS. In one recent report, corneal thickness in patients with PCOS was found to be higher than among controls; this finding was attributed to higher IGF-1 levels in patients with PCOS[29]. It has also been suggested that this association may give rise to more serious eye diseases such as glaucoma[24]. Another research[5] related hyperandrogenism in PCOS patients to decreased tear volume. However none of these studies investigated the broader relation between dry eye and PCOS in terms of IR and hyperandrogenism as well as hematologic parameters.

As well as investigating the effect of hyperandrogenism and insulin resistance on dry eye symptoms, the present article is the first to examine the association between the eye in PCOS patients and novel hematologic markers such as NLR, PLR, and MPV. Increased inflammatory activity and increased NLR has previously been reported in patients with metabolic syndrome[30] and PCOS[31]. The present study also examined this relationship, but no statistical difference was observed between PCOS patients and healthy controls. However, independent of the groups, a negative correlation was found between NLR (which is a useful marker of systemic inflammation) and Schirmer test results.

PLR is a marker associated with adverse cardiovascular events. Increased PLR, has been shown to be another marker of inflammation and a risk factor for formation of platelet-fibrin complexes[32,33]. To our knowledge, the relation between PCOS and PLR has not previously been investigated. In this work, PLR levels did not differ between groups, and no correlation was found between PLR and ocular test results.

MPV, which is an indicator of platelet activation and hypercoagulability, is another new prognostic marker for acute coronary syndrome and cardiovascular diseases[34,35]. In one study[36], MPV values were found to be significantly higher in patients diagnosed with PCOS. The same report noted a positive correlation between MPV values and FG scores, and between MPV values and HOMA-R[36]. In our study, however, MPV scores did not differ between groups. MPV values were also investigated in patients with retinal vein occlusion, which is considered to be associated with vascular damage and hypercoagulability[37-39]. Retinal vein occlusion is reported to be the second most common retinal vascular disease after diabetic retinopathy[37] and there are accumulating data about the increasing levels of MPV in these patients[38,39]. However, there are also articles[37] stating that the values of MPV are lower in these patients and thus is not a useful marker for prediction of this disease. In our research we also failed to demonstrate any statistically significant correlation between MPV and ocular tests investigating dry eye. We believe these new hematologic parameters and their relation to PCOS or ocular diseases require further investigation before any more definitive conclusion can be reached.

The number of PCOS patients with some degree of dry eye but without subjective symptoms

remains unknown. In one study[22], diabetic retinopathy patients with no subjective symptoms of dry eye and normal Schirmer test results showed pathological grades of squamous metaplasia. Our results here show that, although subjective dry eye symptoms (which can be determined by OSDI questionnaire) may not be present in all patients with PCOS, evidence of dry eye can be detected by other sensitive tests in a substantial number of these patients.

One potential limitation of our report was that the study and control population was quite young, with no opportunity to evaluate any long-term effects (i.e., 20-30 years) of PCOS on the eye. This might usefully be the subject of future investigation.

Conclusions

Data concerning ocular changes in patients with PCOS are limited, and dry eye is often overlooked in the context of PCOS. In addition to evaluating the impact of the insulin resistance and hyperandrogenism, we also investigated for the first time the effects of some novel hematologic markers in PCOS and their relation to the tear function. The results showed a correlation between presence of dry eye and hirsutism score (but not the HOMA-R). It was also shown that more sensitive tests can reveal dry eye in a considerable number of asymptomatic patients. Thus, we believe that evaluation of patients with PCOS should include early diagnosis of tear reduction (which can cause further complications) and a careful ophthalmic examination.

Conflict of Interest

We declare that we have no conflict of interest. We state that we have full control of all primary data, and we agree to allow the journal to review our data if requested

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