

Tear osmolarity and tear function assessment in patients with polycystic ovary syndrome

Avaliação da osmolaridade lacrimal e da função lacrimal em pacientes com síndrome dos ovários policísticos

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ABSTRACT

Purpose: Polycystic ovary syndrome (PCOS) is an endocrine disease characterized by chronic anovulation and hyperandrogenism. Hormonal changes can affect tear function. This study evaluates tear function and impact of hyperandrogenism on it in PCOS patients. **Methods:** Fifty patients with PCOS and thirty control volunteers were examined for tear break-up time, Schirmer-I and tear osmolarity. Also, serum levels of total testosterone, FSH, LH and AMH were determined in venous blood samples in the early follicular phase. PCOS patients were divided into two groups by plasma total testosterone level: Group A with normal (≤ 0.513 ng/ml; $n=27$), Group B with higher hormone level (>0.513 ng/ml; $n=23$). Healthy control group indicated as Group C ($n=30$). **Results:** LH, total testosterone levels were higher in the PCOS group than in the control group ($p=0.012$; $p=0.025$). Mean values of tear break-up time and Schirmer-I were different between groups and especially Group A and C were near to each other differing from B ($p>0.05$). Tear osmolarity results were higher in Group B, compared to A and C ($p=0.049$; $p=0.033$). No significant difference detected in tear osmolarity value means of Group A and C ($p=0.107$). AMH levels were higher in Group B, compared to A and C ($p=0.002$; $p=0.001$). AMH levels in Group A were higher than that of C ($p=0.002$). Positive correlation between levels of total testosterone and AMH was detected in all PCOS patients ($n=50$; Pearson's $r=0.579$; $p<0.001$). **Conclusion:** Tear function can be affected in PCOS patients with hyperandrogenism. Tear osmolarity is the most sensitive and objective assessment method for ocular surface changes in PCOS.

Keywords: Polycystic ovary syndrome; Tears/physiology; Osmolar concentration; Hyperandrogenism; Anti-müllerian hormone

RESUMO

Objetivo: A síndrome do ovário policístico (SOP) é uma doença endócrina caracterizada por anovulação crônica e hiperandrogenismo. As alterações hormonais podem afetar a função cardíaca. Este estudo avalia a função lacrimal e o impacto do hiperandrogenismo sobre ela em pacientes com SOP. **Métodos:** Cinquenta pacientes com SOP e trinta voluntárias de controle foram examinadas para tempo de ruptura lacrimal, Schirmer-I e osmolaridade lacrimal. Além disso, os níveis séricos de testosterona total, FSH, LH e HAM foram determinados em amostras de sangue venoso na fase folicular precoce. As pacientes com SOP foram divididas em dois grupos por nível de testosterona plasmática total: Grupo A com nível normal (≤ 0.513 ng/ml; $n = 27$), Grupo B com nível superior de hormônio ($> 0,513$ ng/ml; $n = 23$). Grupo de controle saudável indicado como Grupo C ($n = 30$). **Resultados:** Os níveis de LH e testosterona total foram maiores no grupo com SOP do que no grupo controle ($p = 0,012$; $p = 0,025$). Os valores médios de tempo de ruptura lacrimal e Schirmer-I foram diferentes entre os grupos, e especialmente os Grupos A e C estavam próximos um do outro, diferente do B ($p > 0,05$). Os resultados de osmolaridade lacrimal foram maiores no Grupo B, em comparação com A e C ($p = 0,049$; $p = 0,033$). Não houve diferença significativa detectada em valor médio de osmolaridade lacrimal nos Grupos A e C ($p = 0,107$). Os níveis de HAM foram maiores no Grupo B, em comparação com A e C ($p = 0,002$; $p = 0,001$). Os níveis de AMH no Grupo A foram superiores aos de C ($p = 0,002$). Uma correlação positiva entre os níveis de testosterona total e AMH foi detectada em todas as pacientes com SOP ($n = 50$; Pearson's $r = 0,579$; $p < 0,001$). **Conclusão:** a função lacrimal pode ser afetada em pacientes com SOP com hiperandrogenismo. A osmolaridade lacrimal é o método de avaliação mais sensível e objetivo para alterações da superfície ocular em SOP.

Descritores: Síndrome dos ovários policísticos; Lágrimas / fisiologia; Concentração osmolar; Hiperandrogenismo; Hormônio anti-mülleriano

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine pathology in women of reproductive age and is thought to be one of the leading causes of female subfertility. This is an endocrinopathy which has a prevalence of 4.8 – 8% in women of premenopausal period⁽¹⁻⁴⁾. According to the Rotterdam definition (2003), two of three criteria must be met to diagnose PCOS: chronic oligo and/or anovulation, clinical or biochemical signs of excess androgenic activity and polycystic ovaries (by gynecologic ultrasound), while other entities are excluded that would cause the same⁽⁵⁾.

In this syndrome, serum luteinizing hormone (LH) concentration increases while serum follicle-stimulating hormone (FSH) level often remains the same, and so LH/FSH ratio increases. Also PCOS patients often have insulin resistance and hyperinsulinemia which can result in increased androgen production and decreased levels of sex hormone-binding globulin (SHBG). These all take part in hyperandrogenism pathogenesis⁽⁶⁾. It is known that PCOS patients also have higher serum Anti-Müllerian hormone (AMH) levels than healthy women⁽⁷⁻⁹⁾. Some studies report significant correlations amongst serum AMH and androgens in PCOS⁽⁸⁻¹¹⁾.

Specific receptors for androgens have been identified on the healthy human meibomian and lacrimal glands and conjunctiva, and it is likely that their function is regulated by androgens^(12,15). Dysfunction of any regulation mechanisms can result in evaporative dry eye disease^(1,2,16).

Tear film break-up time (TBUT) and Schirmer I test are the most frequently used tests in diagnosis and follow-up of dry eye disease. Also recently, tear osmolarity measurement has started to be used in clinical evaluation of this eye pathology.

The aim of present study is to estimate and correlate serum levels of testosterone, AMH and LH with ocular surface parameters like tear osmolarity, tear film break-up time (TBUT) and Schirmer I test results in PCOS patients and between them and healthy control group.

METHODS

The study protocol was approved by the local Ethics Committee and performed according to the Helsinki declaration. Written informed consent was obtained from all patients and healthy volunteers. Fifty consecutive patients with recently diagnosed PCOS (study group) and thirty healthy volunteers (control group) were prospectively included in this study. Two groups were comparable in age (Table 1). The diagnosis of PCOS was based on the Rotterdam (2003) criteria⁽⁵⁾. Patients were eligible if they had at least two of three major criteria, including chronic oligo and/or anovulation, clinical or biochemical signs of excess androgenic activity and polycystic ovaries (by gynecologic ultrasound). Exclusion criteria for all subjects included ocular surface disorders such as previous eye surgery, receiving topical or systemic drugs that could affect tearing function, chemical and thermal injuries, Stevens-Johnson syndrome, and ocular cicatricial pemphigoid, current or previous use of contact lens, atopy history, systemic diseases, other endocrinopathies, smoking and drinking alcohol.

From all patients and healthy volunteers venous blood samples were collected in the early follicular phase, between the third and fifth day of the menstrual cycle. Serum levels of hormones, including total testosterone, follicle-stimulating

hormone (FSH), luteinizing hormone (LH) and anti-Müllerian hormone (AMH) concentrations were determined.

Ophthalmic evaluations included, in the following order: complete ophthalmic examination, measurement of tear osmolarity, Schirmer I test without anesthesia and tear film break-up time (TBUT). Each evaluation was performed by an experienced and masked physician in the early follicular phase.

Tear osmolarity was evaluated by the TearLab Osmolarity System (Tearlab, San Diego, CA, USA). This system includes single use test cards containing microchannels to collect tear fluid, a hand-held pen, and a portable reader unit. Tear samples were collected from the inferior lateral tear meniscus after calibration of the system. On slit lamp examination, eyelid margin, meibomian gland orifices, and tarsal and bulbar conjunctiva were evaluated. Basal and reflex tear production was measured by Schirmer I test without anesthesia after slit lamp examination. A standard Schirmer test strip (Haag-Streit UK, Ltd, Harlow, Essex, UK) was placed in the temporal 1/3 part of lower fornix for 5 min, during which time the patient was allowed to blink normally. The filter strip was removed and the length of wetted filter paper determined in millimeter and value recorded. Five minutes later, one drop of 1% sodium fluorescein was instilled to the lower fornix for measurement of tear film break-up time (TBUT). The patient was asked to blink several times to distribute the dye. The patient was then asked to stare directly ahead without blinking. The tear film was then examined with a cobalt blue light on the slit lamp. The time between the last blink and the appearance of the first dry spot in the fluorescein stained tear film was measured in seconds. This procedure was repeated 3 times and the mean value was recorded.

Statistical analysis was performed with SPSS for Windows 17.0 (Statistical Product and Service Solutions, Inc., Chicago, IL, USA) package program and only right eyes were used. In descriptive analysis, the measurement variables are given as mean and standard deviation. The Shapiro-Wilk test was used to identify whether the variables were normally distributed. The differences between groups were assessed by using unpaired t-tests for parametric data. P values of less than 0.05 were considered statistically significant. Pearson's correlation analysis was used to detect correlation between data sets.

RESULTS

Age, plasma hormone levels and ocular surface parameters of PCOS and normal patients are summarized in table 1.

There were no significant difference between mean ages of PCOS and normal group. Mean values of ocular surface parameters were slightly different between PCOS and normal group, but this difference was not statistically significant (unpaired t test; $p > 0.05$). LH, total testosterone levels were significantly higher in the PCOS group than in the control group (unpaired t test; $p = 0.012$ and $p = 0.025$, respectively).

We determined total testosterone cut-off level as 0.513 ng/ml for classifying the PCOS subjects into group A and B in accordance with previous studies⁽¹⁷⁾. We also consider exact hormone level parameters according to patient database of our hospital based laboratories. PCOS patients were divided into two groups by plasma total testosterone level: Group A with normal hormone level (≥ 0.513 ng/ml; 27 PCOS patients) and Group B with higher hormone level (> 0.513 ng/ml; 23 PCOS patients). Normal control group indicated as Group C (30 normal volunteers). Age, TBUT, Schirmer I, tear osmolarity

parameters and plasma AMH levels of patients in these groups were compared and summarized in table 2.

Table 1
Comparison of age, ocular findings and hormone levels in normal and PCOS patients

	PCOS (n=50) Mean ± SD	Control (n=30) Mean ± SD	P value
Age (years)	30.47 ± 6.23	29.22 ± 5.18	0.748
TBUT (sec.)	11.18 ± 5.76	12.92 ± 7.39	0.165
Schirmer I test (mm)	23.15 ± 9.21	26.75 ± 8.06	0.211
Tear osmolarity (mOsm/L)	295.41 ± 12.22	287.32 ± 9.18	0.095
LH (IU/L)	10.17 ± 8.33	4.88 ± 2.56	0.012
FSH (IU/L)	6.01 ± 1.27	6.41 ± 1.53	0.099
Total testosterone (ng/ml)	36.46 ± 1.88	27.97 ± 1.12	0.025
AMH (ng/ml)	9.67 ± 3.19	1.64 ± 0.62	0.083

PCOS, polycystic ovary syndrome; TBUT, tear film break-up time; LH, luteinizing hormone; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone

Table 2
Comparison of ocular findings and AMH level in PCOS patients divided by testosterone level into two groups (Group A and B) and normal patients (Group C)

	PCOS		Control	P values
	Group A (n=27) Mean ± SD	Group B (n=23) Mean ± SD	Group C (n=30) Mean ± SD	
Age (years)	29.41 ± 5.03	32.27 ± 4.26	29.22 ± 5.18	0.661 0.593 0.702
TBUT (sec.)	12.11 ± 4.63	10.12 ± 5.06	12.92 ± 7.39	0.197 0.176 0.208
Schirmer I test (mm)	24.12 ± 7.12	21.18 ± 7.33	26.75 ± 8.06	0.312 0.267 0.401
Tear osmolarity (mOsm/L)	292.31±10.11	301.57±11.19	287.32±9.18	0.049 0.033 0.107
AMH (ng/ml)	4.77 ± 1.19	12.15 ± 2.11	1.64 ± 0.62	0.002 0.001 0.002

Group A - PCOS patients with normal testosterone level (≤0.513 ng/ml); Group B - PCOS patients with increased testosterone level (>0.513 ng/ml); Group C - Normal control group

There were no significant difference between mean ages of all groups. Mean values of tear break up time and Schirmer I test were slightly different between groups and especially Group A and Group C were near to each other differing from Group B. This difference was not statistically significant (unpaired t test; p>0.05).

Tear osmolarity results were significantly higher in Group B, compared to Group A and Group C (unpaired t test; p=0.049 and p=0.033, respectively). No significant difference was detected in tear osmolarity value means between Group A and Group C (p=0.107).

AMH hormone levels were significantly high in Group B, comparing to Group A and Group C (unpaired t test; p=0.002 and p=0.001, respectively). Also, AMH levels in Group A were significantly higher than that of Group C (p=0.002). Medium positive correlation between plasma levels of total testosterone and AMH was detected in all PCOS patients (n=50; Pearson’s r=0.579; p<0.001).

DISCUSSION

In our study, we evaluated tear secretion with objective tests such as Schirmer I, tear film break-up time and tear osmolarity to evaluate the impacts of hormones on ocular surface in PCOS patients.

In our study, mean values of Schirmer I and TBUT values were slightly different between PCOS and normal group, but this difference was not statistically significant (Table 1; p>0.05). Likewise, there were no significant difference between these values obtained from PCOS group with hyperandrogenism, PCOS group with normal androgen levels and control group in our study (Table 2; p>0.05).

We prefer to use objective tests. We don’t use subjective tests such as questioning and scoring symptoms of patients because these tests are not reliable. We don’t use OSDI scores because in our opinion, subjective symptoms recorded by patients generally do not match up with disease severity. Despite the fact that most studies have found weak or no correlations between symptoms and signs of dry eye, we use only objective tests in our study.¹⁸

Tear function tests were used and evaluated in the previous three studies. All these studies compare test results between PCOS patients and healthy control groups except Bonini.¹⁹ In Bonini’s study the results were compared between PCOS and PCO (Polycystic Ovaries - having echographic signs but not clinical or hormonal evidence of hyperandrogenism) patients. Bonini et al., Yavas et al. and Coksuer et al. reported that there were significant difference between TBUT values obtained from PCOS and other groups. In addition to that, there were no significant difference between Schirmer I values obtained from PCOS and other groups in all of the three studies^(19,21).

In our study, we determine the level of total testosterone 0.513 ng/ml to classify PCOS subjects into group A and B according to the previous studies in the literature. Kushnir et al. Reported normal levels of total testosterone in 323 post-menarcheal women to be 9 – 55 ng/dL , with concentrations not being age-dependent throughout all reproductive ages⁽¹⁷⁾. Tear function test results are compared between these groups. Thus, we are able to form opinion about the impact of hyperandrogenism on tear function.

Our results revealed that mean values of tear break up time and Schirmer I test were slightly different between groups but this difference was not statistically significant. In addition, our results revealed a significantly higher tear film osmolarity in PCOS patients with hyperandrogenism compared with normoandrogenic group of them and normal control group (292.31 ± 10.11, 301.57 ± 11.19, 287.32 ± 9.18 mOsm/L; group A, B and C, respectively).

Tear osmolarity results were significantly high in Group B, comparing to Group A and Group C (Table 2; $p=0.049$ and $p=0.033$, respectively). No significant difference detected in tear osmolarity value means of Group A and Group C (Table 2; $p=0.107$).

In previous studies, hyperosmolarity is the only parameter of tearing function quality that can be objectively and reliably measured⁽²²⁾. Osmolarity testing has been declared the “gold standard” of objective dry eye diagnosis and the single best marker of disease severity^(23,24). Our study approve that tear film osmolarity measurement is the most sensitive objective test.

In our study, according to the results obtained from osmolarity test, we suppose that excess androgen levels change the quality and quantity of lipid and mucin layers of tear film. Recent studies have shown that, patients with polycystic ovary syndrome have an increased goblet cell number and MUC5AC mRNA expression, which may contribute to the abnormal mucous discharge⁽¹⁹⁾. In addition, Maters et al. reported a negative correlation between total testosterone and tear function in premenopausal women, whereas total testosterone correlated positively with tear function for women in menopause⁽²⁵⁾.

One recent study, Gonen et al.⁽²⁶⁾ evaluated tear osmolarity test. This study reported that there was no significant difference between the mean tear osmolarity values obtained from PCOS and control groups (285.02 mOsm/L, and 283.30 mOsm/L, respectively; $p = 0.404$).

Gonen et al. reported that most of the clinical and laboratory changes were absent in the recently diagnosed patients with PCOS and does not affect lacrimal and meibomian glands⁽²⁶⁾.

Excess androgenic activity is generally observed in PCOS patients and it is one of the main criteria of PCOS. But the definition is wider, including more patients with or without androgen excess. The findings obtained from the study of patients with androgen excess can not be estimated to patients without androgen excess⁽²⁷⁾.

In our study, we assess AMH levels among PCOS patients and between them and control group. AMH hormone levels were significantly high in Group B, comparing to Group A and Group C (unpaired t test; $p=0.002$ and $p=0.001$, respectively). Also, AMH levels in Group A were significantly higher than that of Group C ($p=0.002$). Significant positive correlation between plasma levels of total testosterone and AMH was detected in all PCOS patients ($n=50$; Pearson's $r=0.579$; $p<0.001$).

AMH, also known as Müllerian inhibiting substance, is a dimeric glycoprotein and a member of the transforming growth factor- β superfamily⁽²⁸⁾. In male, AMH is produced during fetal sex differentiation by sertoli cells and where it induces Müllerian duct inhibition, while in female, it produces only postnatally by granulosa cells from preantral and small antral follicles⁽²⁹⁾. AMH has an inhibitory role in the ovary, and the increased production by granulosa cells may contribute to cessation of follicle development. Therefore AMH level is an important factor for determination of reproductive response to treatment in PCOS patients.

PCOS patients have higher serum AMH levels than normal ones⁽⁷⁻⁸⁾. But the cause of serum AMH level elevation in PCOS is yet unknown; however, this elevation may be a consequence of other factors altered in PCOS, the most obvious being androgen production. Evidence to support this comes from the studies showing that in serum, AMH has been positively correlated to androgen levels^(9, 10, 30).

AMH levels and its effect on tear function were evaluated in women in PCOS for the first time in our study but it is not clear whether ocular surface parameter changes are caused by AMH or testosterone, because AMH and testosterone levels showed significant correlation between each other (Table 2).

CONCLUSION

It is difficult to reveal only one simple underlying mechanism responsible for tear film quality changes in PCOS patients.

But PCOS patients with high androgen and AMH levels are prone to tear film changes and all of them need treatment because of symptoms. In the future, it would be appropriate to follow PCOS patients with high levels of androgen and AMH levels frequently for ocular surface changes. Tear osmolarity test, due to its high reliability and sensitivity should be used in the evaluation of PCOS patients.

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