Objective assessment of ocular surface as an important prognostic factor for dry eye development in patients with glaucoma.

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Abstract

Objectives: To investigate and compare the tear volume, tear film stability, Meibomian gland loss of healthy and glaucoma subjects from the Bulgarian population and to assess the relationship between clinical findings and glaucoma therapy.

Methods: In this observational cross-sectional study, a random cohort of 68 subjects was enrolled and divided into patients with primary open-angle glaucoma or ocular hypertension (POAG/OHT group; n=19) and healthy controls (HC group; n=49). Ocular surface was analysed objectively by Noninvasive tear Break-Up Time (NIBUT), Meibomian gland loss, and Schirmer test for basal secretion. In addition, Central Corneal Thickness (CCT) was measured with pachymetry.

Results: Loss of Meibomian glands (39.85% ± 14.49%), as well as a decrease in Schirmer test values (7.29 mm ± 3.92 mm) and NIBUT (8.26 s ± 4.73 s), were typical in the POAG/OHT group, regardless of disease duration. Significant factors affecting the ocular surface state were age, anti-glaucoma treatment (type and duration), and the presence of preservatives. A worsening of all clinical parameters was evident in the glaucoma patients. A correlation between CCT and NIBUT was established (p=0.037; r=0.167).

Conclusions: Local glaucoma treatment affects the ocular surface objective parameters according to its duration and the composition of treatment eye drops.

Keywords: Meibomian gland loss, NIBUT, Dry eye, Central corneal thickness, Schirmer test.

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Introduction

Dry eye syndrome is a common chronic multifactorial disease that is accompanied by ophthalmic discomfort and changes in visual function [1,2]. Although patients’ sensations are the leading cause for seeking medical treatment, very often they cannot be used to assess the ocular surface state, the severity of the condition or the appropriate management approach [3]. Therefore, some objective tests have been used in the practice of dry eye assessment [4]. These can be divided into tests of tear production, tear stability, and surface damage [3]. The Schirmer test is a common invasive technique used to assess tear production; its variations allow complete measurement of both basal and stimulated secretion [5]. Tear stability is characterized via a number of techniques, including different invasive and noninvasive break-up times [6]. Noninvasive Tear Break-Up Time (NIBUT) is a newly developed technique allowing evaluation of the temporal changes in the tear film between the blinks of the patient [7]. The tear film stability is strongly related to the lipid composition and amount in the tear fluid, as this decrease surface tension. These lipids are mainly produced by sebaceous glands (Meibomian glands) located on the lower and upper eyelid [3]. Abnormal meibum production can be attributed either to absence or deficiency of Meibomian glands, inflammation, neoplasia, or Meibomian gland dysfunction (e.g., related to hyper- or hyposecretion, or obstruction) [8]. Absence or dysfunction of Meibomian glands is very strongly correlated with dry eye [9].

There is a correlation between anti-glaucoma treatment and the development of dry eye syndrome [10-12]. Current research is mainly focused on the effect that the medications, particularly the preservatives contained herein, may exert on the ocular surface [10,12]. The preservatives, especially benzalkonium chloride, are the main factors impairing the normal state and function of the ocular surface [13-15]. Glaucoma represents a heterogeneous group of chronic optic neuropathies, which typically exhibit excavation of the optic nerve head and loss in the visual field [16]. It is considered the primary cause of irreversible blindness worldwide [16]. The most common type
is the Primary Open-Angle Glaucoma (POAG). Changes in Central Cornea Thickness (CCT) have been associated with an increased risk of damage to the optic nerve head and damage in the visual field [17]. In addition, lower CCT values were observed for patients with dry eye [18].

In the present study, noncontact meibography was performed for the first time in glaucoma and ocular hypertension patients in Bulgaria, in order to evaluate factors leading to the development of dry eye disease. The aim of the study was to objectively characterize and compare the tear film stability and Meibomian gland status in glaucoma patients and healthy volunteers in Bulgaria. It was intended to determine main risk factors regarding more successful and suitable glaucoma treatment and simultaneous prevention of dry eye disease progression.

Materials and Methods

Patient selection

The present observational cross-sectional study was in the Department of Ophthalmology at Aleksandrovsk University Hospital in Sofia, Bulgaria. The present study was conducted in accordance with the tenets of the Declaration of Helsinki. Patients were divided into two groups, based on the inclusion and exclusion criteria shown in Table 1. The first group were healthy volunteers (n=49) with no history of ocular disease (healthy control (HC) group). The second group consisted of 19 subjects that had been diagnosed with POAG or ocular hypertension (OHT) (POAG/OHT group).

Study protocol

All patients were subjected to clinical tests of the ocular surface in the following order: NIBUT, Meibography, Schirmer test, and pachymetry, with 5 min intervals between tests. All measurements were performed first on the right eye, and by a single investigator, to minimize inter-examiner bias.

NIBUT measurement

The NIBUT was measured using Oculus Keratograph 5M® (OCULUS, Inc. USA), and recorded in seconds. The evaluation was conducted according to the methodology described by Wiedemann [19]. The procedure was performed in triplicate.

Meibography

Meibomian gland status was investigated by applying noncontact infrared meibography with Oculus Keratograph 5M®, in accordance with the method suggested by Srinivasan [20]. Local anesthetic (Alcaine®, S.A. ALCON, Belgium) was instilled to reduce patient discomfort. Photographs of each eyelid were captured by infrared camera and further analysed by ImageJ 1.50i (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA), as described by Pult and Riede-Pult [21]. The evaluation was performed objectively based on the percentage of the Meibomian Gland Loss (MGL) on each eyelid. The percentage was calculated according the following formula:

$$\text{MGL} (\%) = \frac{A_{\text{drop-out}}}{A_{\text{total}}} \times 100 \rightarrow (1)$$

where $A_{\text{total}}$ is the total area of the eyelid and the $A_{\text{drop-out}}$ is the area where no Meibomian glands can be seen.

Schirmer test

The basal tear secretion was measured using Schirmer test #2 [5] after the application of topical anaesthesia (Alcaine®) to reduce reflex secretion caused by conjunctival irritation from the paper strip and to increase patient compliance five minutes after instillation of the eye drops, a 5 × 35 mm paper strip (I-DEW tear strips, Ophthalmic Experts Ltd., Barking, UK) was folded and gently placed on the temporal one-third of the lower eyelid, bilaterally. During the test, the patient was asked to keep both eyes closed to reduce tear evaporation. The score was recorded as the measured length of wetting from the notch (in mm) after five minutes.

Pachymetry

CCT was measured by Alcon® Ocuscan® RxP pachymeter (Alcon Laboratories, Inc.; Texas; U.S.A) (in µm). Ten measurements with a standard deviation<1 were performed for each eye after applying topical anesthesia (proxymetacaine hydrochloride, Alcaine®). The mean values were used for further analysis.

Statistical analysis

All measured data was subsequently subjected to statistical analysis, including independent samples t-test, ANOVA, Pearson correlation, Spearman correlation, and linear regression using SPSS v. 19.0. A p-value<0.05 was regarded as statistically significant.

Results

Patients

A total of 68 patients (136 eyes) were included in the study. The age and sex distribution of the patients can be seen in Table 2.

A statistically significant difference (p<0.001; df=134) was observed in the mean patient age between the two tested groups.

Schirmer and NIBUT

In order to determine the influence of sex-related factors, Schirmer test and NIBUT values were statistically analysed with independent samples t-tests to compare each of the tested groups. In the healthy group, a statistically significant difference (p=0.003; df: 88) in Schirmer test results was found between men and women. Women exhibit lower values (mean=8.06 mm ± 5.49 mm), compared with men (mean=12.33 mm ± 7.69 mm). In the HC group, women
age>45 y showed lower Schirmer values (7.16 ± 6.32 mm; p=0.015) compared with men at any age. Similar results were found in the POAG/OHT (women age>45 y: 6.14 ± 4.86 mm; men at any age 8.89 ± 5.73 mm; p<0.01).

The age of the patients in the HC group revealed a significant Pearson correlation with the NIBUT values (p<0.037; r=-0.214). A stronger correlation between the same parameters was found in the POAG/OHT group (p<0.018; r=-0.381). In addition, a decrease in the volume and tear film stability was established between the HC group and the POAG/OHT group as can be seen in Figure 1A.

It can be noticed that there is a very strong correlation between NIBUT values and age of the patients in the POAG/OHT groups when patients are divided by gender (p=0.005; r=-0.750). Other age and gender related differences were not established.

**Meibomian glands**

The investigation of the Meibomian glands status in the tested groups was based on analysis of images obtained by the Oculus Keratograph 5M. The total area and the area without Meibomian glands can be seen on the Figure 2.

There was statistical differences between MGL measurements in the POAG/OHT and HC groups for both lower (p=0.029; df: 134) and upper eyelid (p<0.001; df: 134). The results are shown in Figure 1B. Approximately 10% greater total gland loss is detected in the POAG/OHT group (39.85% ± 14.49%), compared with the healthy group (27.10% ± 13.98%).

For the HC group, age was correlated with MGL on the upper eyelid (r=0.271; p=0.007). In the POAG/OHT group, a significant correlation was found only for the lower eyelid (LL) (r=0.331; p=0.042). The total MGL was correlated with age (r=0.219; p=0.031) and NIBUT (p=0.003; r=-0.303) in the healthy group. An independent sample t-test showed that, in the HC group, women age>45 y exhibited a statistically significant decrease in the MGL-LL (26.15 ± 14.61; p<0.01), compare with younger women (14.59 ± 8.91%). As expected, no correlation of Schirmer test results with MGL was established in either of the groups.

**CCT**

The CCT pachymetry results showed some significant differences between the HC and POAG/OHT group (p<0.001). The mean CCT in the healthy cohort was 569.0 ± 30.4 µm whereas in the POAG/OHT group it was 535.9 ± 31.5 µm. In the POAG/OHT group approximately CCT values were 30 µm lower than those in the HC group. Further investigation of the results showed that there was a correlation between the parameter characterizing tear film stability and the CCT in the POAG/OHT group (p=0.037; r=0.167). A regression analysis with confidence interval of 95% was performed.

A correlation was found between the CCT and the patients’ age in the POAG/OHT group (p=0.024; r=-0.386).
LL when beta-blockers were applied. The Schirmer test results were influenced by the API type in cases where carbonic anhydrase inhibitors were used. A significant presence of Benzalkonium Chloride (BAC) was established for the eye drops containing beta-blockers ($p=0.001$) or Carbonic Anhydrase Inhibitors (CAI) ($p=0.045$). The presence of preservative (BAC) in the eye drops showed a significant effect on MGL on the lower eyelid ($p=0.039; r=-0.336$).

**Table 1.** Inclusion/exclusion criteria for study participants (POAG-Primary Open Angle Glaucoma; OHT: Ocular Hypertension; HG: Healthy Group).

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 18-65 y</td>
<td>Age&lt;18 y or &gt;65 y</td>
</tr>
<tr>
<td>Diagnosed with POAG or OHT for the POAG/OHT group</td>
<td>Diagnosed with dry eye disease or self-medicated with artificial tears</td>
</tr>
<tr>
<td>Healthy volunteers (HG)</td>
<td>Refractive surgery history</td>
</tr>
<tr>
<td></td>
<td>Application of contact lenses</td>
</tr>
<tr>
<td></td>
<td>Other ocular disease or application of eye drops other than for treatment of POAG or OHT</td>
</tr>
<tr>
<td></td>
<td>Atopia or allergic disease</td>
</tr>
</tbody>
</table>

**Table 2.** Descriptive statistics of the tested subset of populations (HG: Healthy Group; POAG/OHT: Primary Open Angle Glaucoma/Ocular Hypertension group).

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>%</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25</td>
<td>51.0</td>
<td>43.16</td>
<td>15.06</td>
<td>21.0</td>
<td>65.0</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>49.0</td>
<td>42.13</td>
<td>12.62</td>
<td>21.0</td>
<td>64.0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>100.0</td>
<td>42.65</td>
<td>13.85</td>
<td>21.0</td>
<td>65.0</td>
</tr>
</tbody>
</table>

**Table 3.** Pearson correlation analysis in the POAG/OHT group (MGL-UL Meibomian Gland Loss-Upper Eyelid; MGL-LL Meibomian Glands Loss-Lower Eyelid) (significant correlations are marked in red).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MGL-LL (%)</th>
<th>NIBUT (s)</th>
<th>Schirmer (mm)</th>
<th>CCT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HG</td>
<td>POAG/OHT</td>
<td>HG</td>
<td>POAG/OHT</td>
</tr>
<tr>
<td>MGL-UL (%)</td>
<td>$r=0.427$</td>
<td>$r=0.130$</td>
<td>$r=0.181$</td>
<td>$r=0.423$</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
<td>$p=0.436$</td>
<td>$p=0.077$</td>
<td>$p=0.008$</td>
</tr>
<tr>
<td>MGL-LL (%)</td>
<td>$r=1$</td>
<td>$r=0.327$</td>
<td>$r=0.034$</td>
<td>$r=0.025$</td>
</tr>
<tr>
<td></td>
<td>$p=0.001$</td>
<td>$p=0.838$</td>
<td>$p=0.818$</td>
<td>$p=0.545$</td>
</tr>
<tr>
<td>NIBUT (s)</td>
<td>$r=1$</td>
<td>$r=0.307$</td>
<td>$r=0.080$</td>
<td>$r=0.151$</td>
</tr>
<tr>
<td>Schirmer (mm)</td>
<td>$r=1$</td>
<td>$r=0.083$</td>
<td>$r=0.135$</td>
<td>$p=0.698$</td>
</tr>
</tbody>
</table>

**Table 4.** Correlation coefficients and $p$-values for the main tested parameters and treatment characteristics (API: Active Pharmaceutical Ingredient; BAC: Benzalkonium Chloride; CAI: Carboanhydrase Inhibitor).

<table>
<thead>
<tr>
<th>Investigated parameter</th>
<th>Treatment duration</th>
<th>Number of API</th>
<th>Type of API</th>
<th>BAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGL-UL (%)</td>
<td>$r=-0.193$</td>
<td>$r=-0.042$</td>
<td>$p&gt;0.05$</td>
<td>$r=0.168$</td>
</tr>
<tr>
<td></td>
<td>$p=0.247$</td>
<td>$p=0.803$</td>
<td>$p=0.313$</td>
<td></td>
</tr>
<tr>
<td>MGL-LL (%)</td>
<td>$r=-0.211$</td>
<td>$r=-0.231$</td>
<td>$r=-0.373$</td>
<td>$r=-0.336$</td>
</tr>
<tr>
<td></td>
<td>$p=0.205$</td>
<td>$p=0.163$</td>
<td>$p=0.021$ (β-blocker)</td>
<td>$p=0.039$</td>
</tr>
</tbody>
</table>
Objective assessment of ocular surface as an important prognostic factor for dry eye development in patients with glaucoma

<table>
<thead>
<tr>
<th>Schirmer (mm)</th>
<th>NIBUT (s)</th>
<th>CCT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r=0.107</td>
<td>r=0.334</td>
<td>p=0.034</td>
</tr>
<tr>
<td>p=0.546</td>
<td>r=0.027</td>
<td>p=0.053</td>
</tr>
<tr>
<td>r=0.138</td>
<td>p=0.040</td>
<td>p=0.378</td>
</tr>
<tr>
<td>r=0.483</td>
<td>p&gt;0.05</td>
<td>p=0.879</td>
</tr>
<tr>
<td>r=0.030</td>
<td>r=0.087</td>
<td>p=0.027</td>
</tr>
<tr>
<td></td>
<td>p=0.605</td>
<td>p=0.879</td>
</tr>
</tbody>
</table>

Discussion

There are few other studies investigating dry eye disease by simultaneously using MGL, NIBUT and Schirmer test for basal secretion in dry eye patients [7,8,10,19,20]. According to the available literature, there are no other studies using all of these objective tests for dry eye assessment in glaucoma patients in Bulgaria. The subjects in the present study were selected based on a wide age range, as it was necessary to determine if the changes in the clinical findings were related to patient age. The upper limit (65 y) was selected following the World Health Organization definition of elderly people [22]. The elderly are usually co-morbid and some of the changes in their ocular status could be a result of a variety of systemic diseases. Therefore, they were not included in the study. In addition, there is data showing that 15% of the people older than 65 y suffer from dry eye disease [23]. Furthermore, the patients included in the POAG/OHT group were either newly diagnosed or have been treated for glaucoma for many years. A difference was found concerning the mean age in the two groups. The main reason for the higher age of the patients from POAG/OHT group, compared with the HC group, is that glaucoma and ocular hypertension are age-related diseases and prevail in patients older than 45 y [24]. This age difference could lead to some bias in the data comparison between the two groups. The upper limit set in our exclusion criteria helped to decrease the influence of age on the results. Furthermore, it is necessary to include patients with various treatment durations regarding the influence of therapy on POAG/OHT. The prevalence of women in the POAG/OHT group might be explained by an increased willingness for evaluation.

Dry eye disease is predominantly associated with women [25]. The women in our HC group were characterized with lower Schirmer test values than the men in the same group. This might be a result of the sex-related differences in the lacrimal glands [25,26]. In the present study, no significant difference in tear secretion was found between men and women in the POAG/OHT group. However, it was evident that women age>45 y presented lower Schirmer test values than younger women or men at any age. This could be explained by hormonal changes that occur after menopause.

The Spearman correlation in this study showed that the type of API and the presence of BAC are related to the loss of Meibomian glands on the lower eyelid. MGL on the upper eyelid is better correlated to the stability of the tear film, in contrast with MGL on the lower eyelid. Similar results have been reported by Pult et al. [32].

Topical glaucoma treatment has a significant versatile effect on the ocular surface. The application of β-blockers induces Meibomian gland alterations. The instillation of CAIs affects basal tear production. Thus, long-term treatment contributes to a decrease in NIBUT. In the present study, BAC-containing eye drops had a significant effect on the Meibomian glands. Our results partially confirm previously reported data [33]. Glaucoma therapy seems to be one of the most critical factors.
influencing the surface state in dry eye. This highlights the complexity of the problem and shows that the clinical findings continue to be inconsistent between studies [34]. It is, therefore, very important to choose suitable anti-glaucoma therapies with regard to the state of the ocular surface.

Additionally parameters typically tested for glaucoma include CCT, were evaluated in relation to ocular surface parameters. In the present study, a correlation was found between CCT and the NIBUT in the POAG/OHT group; however, a similar observation was not present for the HC group. A previous study [26] reported a correlation between dry eye disease and thinner central cornea in women. Nevertheless, a correlation therefore, very important to choose suitable anti-glaucoma therapies with regard to the state of the ocular surface.

References

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