Investigation of the Effect of Smoking and Schirmer Test Scores on Ocular Demodex Colonization in Healthy Middle-Aged Adults

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Abstract

Purpose: To determine whether cigarette use and Schirmer test score difference in middle-aged individuals without chronic illness and ocular complaints leads to changes in ocular Demodex colonization.

Methods: Healthy adults without chronic disease and ocular complaints between 40-68 years old with visual impairment (presbyopia) were included. After detailed ophthalmologic evaluation Schirmer test was performed and two eyelashes were taken from the two eyelids of lower cover, two drops of physiological saline were placed between the lamellae and analyzed in the microbiology laboratory.

Results: 102 eyes of 102 patients were included in the study. 55 were men (53.9%) and 47 were women (46.1%). The mean age of the males was 52.50 ± 6.1 while the mean age of the females was 50.85 ± 6.1 years. Demodex presence was statistically significant (chi square p=0.04) when the Schirmer score was below 5 and 5, Schirmer score and the number of Demodex was lower than 5 and 5 (Mann-Whitney U p: 0.03), the Schirmer score of smokers was statistically significantly lower (Mann-Whitney U p: 0.03). There was no relationship between Demodex presence (chi square p=0.402) and Demodex number (Mann-Whitney U p: 0.81) among smokers.

Conclusion: It was observed that Schirmer score reductions in individuals without any systemic disease and ocular complaints may be related to the presence of Demodex, and smoking among asymptomatic individuals reduced Schirmer test scores.

Keywords: Schirmer test; Ocular Demodex colonization; Ectoparasites; Presbyopia

Introduction

Ectoparasites in Demodex species are many mammalians infecting organisms. Despite being described in the middle of the 19th century, these ectoparasitic clinical findings have attracted attention in recent years by ophthalmologists and dermatologists. The human eye is not as easily accessible as the rest of the body for hygiene reasons because of its sheltered anatomic position. Therefore, ocular Demodex infestation is thought to be caused by facial skin infestation [1]. Demodex folliculorum has long opistosomes and it is seen in groups of hair follicles in the length of 0.3-0.4 mm; Demodex brevis, 0.2-0.3 mm in length, is found in sebaceous gland tissue alone [2-5]. Therefore, D. folliculorum is detected more frequently than D. brevis in the analysis of eyelash specimens. The life span of Demodex mite from egg to adulthood lasts 3-4 weeks, and females can live 5 days after ovulating. Demodexes are fed on epidermal skin cells and sebum, so they live in the cheeks, nose, chin and periorcular areas, the most common areas of sebaceous glands [5,6]. Investigations have reported that D. folliculorum and D. brevis are associated with blepharitis [7,8] and chalazia [9-12] (Figure 1)

The prevalence of Demodex species was determined in the majority of studies performed in diseased related groups, but no study between the smoking use and the Demodex relationship was found. The aim of this study was to determine the prevalence of mites in the eyelashes of healthy people, to determine the prevalence of smoking and Schirmer 1 test scores in Demodex and how it affects Demodex existence and number.

Methods

This observational cross-sectional study was carried out between January 2017 and January 2018, at Ahi Evran University, who admitted to clinic of the eye diseases with the complaint of presbyopia. The study was performed on healthy 40-68-year-olds who did not have systemic disease and ocular complaints. Ahi Evran University ethics committee was granted for the study. The study was conducted in accordance with the Helsinki Declaration on Good Clinical Practice and all patients provided written informed consent. After detailed ophthalmologic evaluation, eyesight-free visual acuity was 20/20 or more, intraocular pressure was between normal values (10-21 mm/hg), no pathology was found in the evaluation of anterior segment and posterior segment, patients who were not under any systemic and topical treatment were separated as smokers and non-smokers.

The variables used in the study were age, gender, Schirmer 1 test (non-anesthesia mm/5 min) score, Demodex presence, Demodex...
type (*D. brevis*, *D. folliculorum*), Demodex number, smoking history, smoking year.

**Sample Collection and Analysis**

Immediately after the near and distant visual examinations, tear analysis was performed with the Schirmer 1 test method, which is a basal and reflex tear measurement method and no anesthetic agent. It was recorded in mm after waiting for 5 minutes.

Two eyelashes, which were taken from both lower eyelids with the aid of collet, were placed on 2 drops of saline solution, and two coverslips were placed on them and immediately taken relevant expert to analyzed in the microbiology laboratory. Blindness was provided because the analyst did not have the demographic information of the owners of the lashes they analyzed.

**Results and Discussion**

102 eyes of 102 patients were included in the study. 55 were men (53.9%) and 47 were women (46.1%). The mean age of the males was 52.50 ± 6.1 while the mean age of the females was 50.85 ± 6.1 years. Kolmogorov smirnov test was performed with normality analysis. Non-parametric tests were applied because Demodex number (p=0.000), smoking years (p=0.000) and Schirmer 1 test scores (p=0.002) did not fit the normal distribution. Age remained normal distribution (p=0.35).

When the groups included in the study were compared in terms of Demodex numbers; while the relationship between smokers and non-smokers (Mann-Whitney U p: 0.81), the relationship between male and female gender (Mann-Whitney U p: 0.77), relationship between age 55 and older and those aged 54 years or less (Mann-Whitney U p: 0.13) was not statistically significant, The correlation between the values of the Schirmer 1 test score of 5 mm/min and those above 6 mm / min (Mann-Whitney U p: 0.03) was statistically significant (Table 1).

When the groups included in the study were compared in terms of Demodex positivity and negativity are examined in types (*D. brevis*, *D. folliculorum*), Demodex number, smoking history, smoking year.

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When the groups included in the study were compared in terms of Demodex positivity and gender (chi square p: 0.56), the relationship between male and female gender (Mann-Whitney U p: 0.03) was statistically significant, the relationship between male and female gender (Mann-Whitney U p: 0.25), the relationship between age 55 years and older and those aged 54 years or less (Mann-Whitney U p: 0.49) and the relationship between Demodex positive and Demodex negative (Mann-Whitney U p: 0.18) were statistically insignificant (Table 2).

When Demodex positivity and negativity are examined in terms of categorical variables; The relationship between Demodex positivity and gender (chi square p: 0.56), the relationship between Demodex positivity and age 55 (chi square p: 0.12), the relationship between Demodex positivity and smoking (chi square p: 0.40) was not statistically significant, while the relationship between Demodex positivity and Schirmer 1 score 5 mm/min border (chi square p: 0.04) was statistically significant (Table 3).

**Conclusion**

The role of *Demodex* mites in the pathogenesis is discussed in the long-term because of the increasing prevalence of age-related prevalence and frequent occurrence in asymptomatic individuals [13-16]. Furthermore, the relationship between *Demodex* number and blepharitis has not been convincing [1]. Ocular *Demodex* model could not be developed in animals because people are the only hosts for *Demodex*.

Kemal et al. found prevalence of *Demodex* in 28.8% of blepharitis patients and 26.7% of *Demodex* in healthy nonblepharitis subjects [15]. In our study, *Demodex* positivity rate was 34.3% in healthy adults aged 40-68 years who had no ocular or systemic disease except smoking. *Demodex* positivity was 36.8% in non-smokers whereas this rate was 31.2% in smokers (smoker-nonsmoker, chi square p=0.4). *Demodex* positivity was 34.5% in male gender and 34% in female gender (male-female gender, chi square p=0.56). *Demodex* positivity over 55 years of age was 43.7%, while 30% at 54 years and under (55 years old and over 54 years old and p=0.12). *Demodex* positivity was 48.5% when the Schirmer 1 test score was less than 5 mm/min, while it was 26.8% when the schirmer 1 test score was above 6 mm/min (schirrmer 1 5 mm/min and lower schmider 1 6 mm/min over, chi square p=0.04).

Lee et al. a study in which they investigated *Demodex* frequency and ocular discomfort linkage, showed a significant correlation with age, sex, and tear break time, but there was no significant relationship with the schirmer test scores [16]. Patients included in our study do not have systemic discomforts, and there are no ocular complaints. Although Schirmer 1 test scores were below 5 mm/min in healthy subjects without ocular complaints, we showed a statistically significant

<table>
<thead>
<tr>
<th>Schirmer 1 score (mm/5 dk.)</th>
<th>In smokers (n=44)</th>
<th>p: 0.03 (Mann-Whitney U)</th>
<th>In smokers (n=44)</th>
<th>p: 0.03 (Mann-Whitney U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 package per day (n=39)</td>
<td>7.53 ± 3.97 (3-20)</td>
<td>p: 0.03 (Mann-Whitney U)</td>
<td>1 package per day (n=5)</td>
<td>8.00 ± 4.00 (3-13)</td>
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<tr>
<td>1 package per day (n=5)</td>
<td>8.00 ± 4.00 (3-13)</td>
<td>p: 0.03 (Mann-Whitney U)</td>
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<tr>
<td>Non-smokers (n=58)</td>
<td>9.29 ± 4.50 (3-20)</td>
<td>p: 0.25 (Mann-Whitney U)</td>
<td>Female (n=47)</td>
<td>9.10 ± 4.66 (3-20)</td>
</tr>
<tr>
<td>Male (n=55)</td>
<td>8.09 ± 4.01 (3-17)</td>
<td>p: 0.25 (Mann-Whitney U)</td>
<td></td>
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<tr>
<td>55 years and over (n=32)</td>
<td>9.09 ± 4.61 (3-20)</td>
<td>p: 0.49 (Mann-Whitney U)</td>
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<tr>
<td>54 years and under (n=70)</td>
<td>8.31 ± 4.21 (3-20)</td>
<td>p: 0.18 (Mann-Whitney U)</td>
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<tr>
<td>Schirmer positive (n=35)</td>
<td>7.78 ± 4.22 (3-17)</td>
<td>p: 0.40 (Mann-Whitney U)</td>
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<tr>
<td>Demodex Brevis (n=9)</td>
<td>9.66 ± 5.26 (3-17)</td>
<td>p: 0.04 (Mann-Whitney U)</td>
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<tr>
<td>Demodex folliculorum (n=26)</td>
<td>7.23 ± 3.79 (3-16)</td>
<td>p: 0.04 (Mann-Whitney U)</td>
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<tr>
<td>Demodex negative (n=67)</td>
<td>8.92 ± 4.34 (3-20)</td>
<td>p: 0.04 (Mann-Whitney U)</td>
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</tr>
</tbody>
</table>

Table 2: Schirmer 1 score (mm/5 dk.).

<table>
<thead>
<tr>
<th>Demodex positive</th>
<th>Demodex Negative</th>
<th>Chi Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male n=19</td>
<td>Male n=36</td>
<td>p: 0.56</td>
</tr>
<tr>
<td>Female n=16</td>
<td>Female n=31</td>
<td>p: 0.56</td>
</tr>
<tr>
<td>55 years and over n=14</td>
<td>55 years and over n=18</td>
<td>p: 0.12</td>
</tr>
<tr>
<td>54 years and under n=21</td>
<td>54 years and under n=49</td>
<td>p: 0.12</td>
</tr>
<tr>
<td>In smokers n=14</td>
<td>In smokers n=30</td>
<td>p: 0.40</td>
</tr>
<tr>
<td>Non-smokers n=21</td>
<td>Non-smokers n=37</td>
<td>p: 0.04</td>
</tr>
<tr>
<td>Schirmer 1 score lower than 5 and 5 n=17</td>
<td>Schirmer 1 score lower than 5 and 5 n=18</td>
<td>p: 0.04</td>
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<tr>
<td>Schirmer 1 score higher than 6 and 6 n=18</td>
<td>Schirmer 1 score higher than 6 and 6 n=49</td>
<td>p: 0.04</td>
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Table 3: Comparison.
relationship with Demodex presence and Demodex number. Despite the significant significance of Schirmer test scores in smoking smokers, we found no significant association with Demodex presence and number in smokers.

When we look at the literature with Demodex, we see that the number of eyelashes taken from studies done in a wide range of eyelashes ranging from only 2 eyelashes from each eye to 4 eyelashes from one eyelid [17-19]. Since our patients who were included in our study were asymptomatic and we aimed to demonstrate the presence of Demodex existence rather than Demodex number, we got 4 eyelashes in total, 2 in each eye. However, after the samples were instilled with saline, they were quickly moved to the microbiology laboratory, which is located next to the policlinic of eye diseases and because it was evaluated by an experienced microbiologist and parasitologist in Demodex, we also found positive results at high densities, such as 11 Demodex in 4 lashes.

The number of studies showing an association with ocular Demodex presence schirmer test scores is extremely low, we think that this study is valuable in terms of contribution to the literature since the studies investigating the relationship with smoking are not as far as we know it.

The weakness of the study is that more than one sample may be taken from the lower and upper eyelids, but we think that it will create a serious comfort problem for the patient in spite of the studies claiming that 8 clasps are taken from one eye.

The references are: