Abstract
Purpose: To assess the relationship between the meibomian gland loss (MGL) and relevant ocular surface clinical parameters and the influence of age in its relationship. Methods: A total of 161 participants (mean age; 42±17 years) were enrolled in this study. Infrared meibography was performed using Keratograph 5M (K5M; Oculus GmbH, Wetzlar). Participants were divided into five groups according to total meiboscore and the ocular surface parameters of each MGL group were studied. In addition, the relationship between MGL and the ocular surface parameters was established including age as covariant. Results: Both eyelids were taken into account since no association between the MGL from upper and lower eyelid was found (k value=0.2; p=0.3) despite they were significantly correlated (r= 0.3; p<0.001). No statistically significant differences were found in symptomatology among different MGL groups. Statistically significant differences were found among MGL groups in tear osmolarity (p=0.02), bulbar redness (p=0.04), corneal and conjunctival staining (p=0.01 and p=0.004, respectively). Despite it, only corneal staining showed a significant correlation with MGL when age was covariant (r=0.2; p=0.04). Conclusions: MGL higher than 50% seems to be accompanied by signs on the ocular surface. Furthermore, age demonstrated to be a relevant factor when assessing the MGL. For this reason, future studies should compare matched-age groups in order to know the contribution of the MGL on the ocular surface and establish valid cut-off values for dry eye diagnosis.

Keywords
Meibomian gland loss; Infrared Meibography; Ocular surface; Tear film; Symptomatology; Age

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Table 5.docx [Table]
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Highlights

- Meibomian gland loss (MGL) higher than 50% is accompanied by signs in the ocular surface.
- Age is a highly influential factor that must be taken into account when assessing the MGL.
- Only corneal staining was correlated to MGL when age was covariant.
Title
THE INFLUENCE OF MEIBOMIAN GLAND LOSS ON OCULAR SURFACE
CLINICAL PARAMETERS

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1. INTRODUCTION

Dry eye disease (DED) understanding has grown over the last three decades. This multifactorial disorder is characterised by a loss of homeostasis of the tear film and accompanied by ocular symptoms, neurosensory abnormalities, ocular surface inflammation and damage.[1] DED prevalence ranges between 5% and 50% worldwide[2] and it increases linearly with age which makes DED a growing public health issue as the global population of older people is expected to be more than double its current amount by 2050.[3] According to TFOS DEWS II DED classification, the aqueous deficiency (ADDE) and evaporative dry eye (EDE) are the two major types of DED and are considered to exist on a continuum rather than as separate entities.[4] It has been reported that the evaporative component is more common, making EDE the most common type of DED.[2] Meibomian gland dysfunction (MGD) has been considered the major cause of EDE.[5] Changes in the quantity and quality of the meibomian gland (MG) secretion lead to an unstable lipid layer of the tear film, provoking an increase of the evaporation of the underlying aqueous layer.[6] Therefore, any change that occurs in the structure or function of MG could have an important clinical impact.

Currently, non-contact infrared meibography (NIM) is widely used to assess MG non-invasively[7–9]. Several scoring systems for assessment of the meibomian gland loss (MGL) or dropout [7,10–14]have been proposed. The meiboscore proposed by Arita et al. [7] is one of the most commonly used scoring system for MGL evaluation. Previous studies found significant correlations between MGL and some tear film parameters (such as tear film break-up time (TBUT) [15–17], non-invasive tear film break-up time (NIBUT), [18] lipid layer thickness (LLT) [19], Schirmer test [20], MG secretion quality [16] and corneal staining [17]) as well as
subjective symptomatology (Ocular Surface Disease Index (OSDI) [10] and Mcmonnies [21] questionnaires), suggesting its possible diagnostic value.[22] However, others studies concluded that assessing MGL alone as clinical parameter has not enough DED diagnostic value, and that it should be interpreted together with other clinical parameters.[16,23,24] Furthermore, it has been well-documented that MGL increases with age in both healthy subjects [7,15,25] and DED patients.[26] Indeed, a significant positive correlation between age and MGL was detected, indicating that the number of MG decreases with age.[7] On the other hand, in a previous study it has been observed that several ocular surface parameters such as ocular redness, corneal and conjunctival staining are highly influenced by ageing.[27] As well, TFOS DEWS II recently underlined that clinical DED signs increase in a higher amount by decade compared with symptoms.[2] These findings highlight the role of age in both MGL and ocular surface parameters. Thus, the aim of this prospective study was to assess the relationship between MGL and ocular surface parameters and the role of age on its relationship.
2. MATERIAL AND METHODS

2.1 Participants

One-hundred sixty-one participants were included in this prospective study. Participants were recruited via email notices sent to the institutional e-mails from the academic community and advertisements placed on noticeboards at the university. All examinations were completed in the Faculty of Optics and Optometry at Complutense University of Madrid. This study was reviewed and approved by the Ethics Committee of San Carlos University Hospital (Madrid, Spain) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all included participants after explanation of the purpose and possible consequences of the study. Participants were required to be 18 years of age or older, be able to complete the questionnaires, understand the procedures and obtain an evaluable meibography image of the upper (UL) and lower (LL) eyelids. On the other hand, participants were excluded if they had a history of any active ocular disease different from DED and MGD (corneal ulcers, herpes simplex, keratitis...), any uncontrolled severe systemic disease that may affected the eye (Sjögren syndrome, diabetes type II, dermatological diseases...) or any ocular surgery or trauma that could affect the tear distribution and any eyelid margin abnormality. Contact lens wearers were accepted but they were required not to use their contact lenses within the week before performing the clinical examination.

2.2 Study Protocol

All measurements were accomplished by the same examiner and performed from the least to the most invasive in order to minimize the effect of the previous
measurement. Only the right eye (RE) of each participant was assessed (see Figure 1).

2.2.1 Symptomatology Assessment

Participants were required to complete two of the most common DED Questionnaires during the examination: The OSDI questionnaire [28] and the Standard Patient Evaluation of Eye Dryness (SPEED) questionnaire.[29]

2.2.2 Tear Film Osmolarity

Tear film osmolarity (TFO) was measured using the TearLab Osmolarity System (TearLab Corp, San Diego, CA, USA) according to the manufacturer’s instructions. It was conducted in first place in order to avoid reflex tearing or the instillation of any dye that could affect the results.

2.2.3 Keratograph 5M Automated Measurements

Automated measurements were performed using the Keratograph 5M (K5M; Oculus GmbH, Wetzlar, Germany) equipped with the modified tear film scanning function. Three measurements of the tear meniscus height (TMHk), first tear film break-up time (NIKBUT-fr), the average time of all tear film break-up time incidents (NIKBUT-avg), bulbar redness (BR) and limbal redness (LR) were obtained automatically with Oculus K5M software according to the manufacturer’s instructions.

2.2.4 Slit-Lamp Examination

Slit-lamp examination of the cornea and conjunctiva was performed under diffuse illumination using x10 – x16 magnification. Corneal integrity was assessed by instilling a fluorescein dye. Two minutes later, corneal staining was graded using the Oxford scoring scheme.[30] TBUT was measured three times with a
stopwatch and was averaged for the subsequent analysis. Furthermore, bulbar conjunctival integrity was assessed using lissamine green and graded using the Oxford scoring scheme. [30] The meibum quality expression from the central 8 MG was assessed on a scale from 0 to 3: 0 = clear meibum readily expressed; 1= cloudy meibum expressed with mild pressure; 2=cloudy meibum expressed with more than moderate pressure; 3= meibum could not be expressed even with strong pressure.

2.2.5 Keratograph 5M Infrared Meibography

NIM was performed in order to assess MG morphology of the UL and LL of each participant. MGL of the UL and LL was graded subjectively by an experienced examiner using the meiboscore introduced by Arita et al (grade 0, no gland loss; grade 1, area of gland loss <33%; grade 2, area of gland loss 33%–67%; and grade 3, area of gland loss >67%). [7] The meiboscore for each eyelid was summed to give a total score between 0 and 6 (or from 0 to 6). This total meiboscore was used to divide participants into five groups according with the amount of MGL (see Figure 2). In this regard, the group 1 was constituted by participants who presented a total meiboscore of 0; group 2 by participants who showed a total meiboscore of 1; group 3 by participants who showed a total meiboscore of 2; group 4 by participants who showed a total meiboscore of 3 and group 5 by participants who showed a total meiboscore of 4,5 or 6. According the groups established, the percentage range of MGL was 0, 0-16.6, 16.5-33,33-49.5 and >50, for groups 1, 2, 3, 4 and 5 respectively.

2.2.6 Tear Film Volume

Schirmer's test was performed with topical anaesthesia (Colirio Anestésico Doble®, Alcon Laboratories, Spain) and was the final test carried out in the
examination protocol. One drop of topical anaesthesia was instilled on the conjunctival lower fornix of the RE, 5 minutes prior to do the test. Then, the Schirmer strip (35-mm Whatman filter paper; Tiedra Laboratories, Spain) was placed in the lower conjunctival sac at the junction of the lateral and middle thirds (avoiding contact with the cornea) and after 5 minutes the length of wetting was recorded. Participants were seated at rest and were asked to close the eyes during the test.

2.3 Data Analysis

The values are expressed as means ± standard deviation (SD) and the significance level was set p< 0.05 with 95% of confidence level. Normality of the data distribution was tested using the Kolmogorov–Smirnov test. Accordingly, Cohen’s kappa coefficient (weighted kappa value- 95% of confidence) was calculated and classified as follow: 0.00(poor), 0.00-0.20 (slight), 0.21-0.40 (fair), 0.41-0.60 (moderate),0.61-0.80(substantial) and 0.80-1.0 (close to perfect) [31]. Kruskal–Wallis test was used for comparisons between different MGL groups. When statistically significant differences were found (p<0.05), post hoc tests were performed for multiple comparisons applying Bonferroni corrections. Correlation coefficients between MGL of both eyelids and ocular surface parameters were calculated with the Spearman correlation coefficient. In addition, partial correlation was performed including age as a covariant. These correlations were considered strong if they were >0.80, moderately strong if they were between 0.5 and 0.8, fair if they were within the range of 0.3 and 0.5 and poor if they were < 0.30.[32]
3. RESULTS

The demographic characteristics of the participants are summarized in Table 1. From the 161 participants 91 were females and 70 males. Age range among participants was 19 to 88 years with a mean age of 42±17 years.

3.1 Association and correlation between UL and LL

A contingency table was used to compare MGL between UL and LL (Table 2) in order to know if it is necessary to evaluate the MGL in one eyelid or in both. Weighted kappa statistics showed no statistically significant agreement (weighted k value=0.2; p=0.3; range 0.099-0.353, 95% confidence limit) indicating no association between both eyelids. Despite it, a fair and statistically significant correlation was found between UL and LL (Spearman: r= 0.3; p<0.001). For this reason, both eyelids should be assessed in order to know the overall condition of MG and its possible influence in the ocular surface state.

Table 3 shows the demographics of the participants as a function of total Meiboscore.

3.2 Symptomatology assessment

Results regarding subjective symptomatology are showed in Table 4. No statistically significant differences were found in OSDI (p=0.3) and SPEED (p=0.506) questionnaires among different MGL groups.

3.3 Classical Clinical Parameters

Classical clinical parameters results for each group are shown in Table 5. Statistically significant differences were found among groups in TFO (p=0.02), corneal (p=0.01) and conjunctival staining (p=0.004). For TFO, there were statistically significant differences between group 5 and groups 1,2 and 3 (p=0.02; p=0.01; p=0.001, respectively). For corneal staining, there were statistically
significant differences between group 5 and groups 1 and 2 (p=0.04 and 0.003, respectively). Regarding conjunctival staining, there were also statistically significant differences between group 5 and groups 1 and 2 (p=0.02 and 0.007, respectively) and between group 4 and groups 1 and 2 (p= 0.04 and 0.01, respectively).

3.4 Keratograph 5M Automated Measurements

Table 6 shows the automated measurements obtained with K5M for each meiboscore group. Statistically significant differences in BR were found between groups 5 and 1 (p=0.04) and groups 5 and 2 (p=0.01).

3.5 Relationship between MGL and ocular surface parameters considering age as covariant.

Partial correlations between MGL and ocular surface parameters are shown in Table 7. No relationship was found between MGL and any ocular surface parameter except for corneal staining (r=0.2, p=0.04) when age was included as covariant.
4. DISCUSSION

NIM has been proven to be a useful technique for non-invasive observation of MG morphology in order to help physicians improve DED diagnosis and treatments.[7,33,34]

The aim of the study is to assess the correlation between MGL and some other ocular surface parameters. Accordingly, first it has to be determined if both (UL and LL) or only one eyelid should be included for MGL assessment. This matter is quite controversial since different studies with different aims have considered both or only one eyelid. Routinely, LL is the most commonly assessed due to its accessibility which provokes less discomfort to the patient. It is believed that comparable outcome can be expected for MGL assessment choosing one or two eyelids, as UL and LL MGL seem to be significantly correlated.[18] Dogan et al. [35] assessed meibography images of 30 patients and proposed to evaluate only the UL for MGL assessment because of its correlation with TBUT and better inter-examiner agreement on MGL. On the other hand, Finis et al. [16] performed a retrospective analysis of 128 patients and found a strong correlation between meiboscores of the UL and the LL as well as with the total meiboscore. This suggests that MGL assessment based on the evaluation of the LL might be enough for the clinical routine. In the present study a positive and fair statistically significant correlation between MGL of UL and LL was found. However, there was no agreement as revealed by the Kappa statistic, suggesting that, despite there exist a relationship between MGL of both eyelids there is a bias in their meiboscore. Our results could support those found by Pult et al. [18], who suggested the assessment the MGL of both eyelids.
After concluding that both eyelids should be taken into account in order to assess MGL, participants were classified into five groups according to the total meiboscore.\textsuperscript{[7]}

DED symptomatology was evaluated using OSDI and SPEED questionnaires in the current study. No statistically significant differences were found in DED questionnaires among different MGL groups. However, as Table 4 shows, the symptomatology score for group 5, in both questionnaires, is clinically higher than for the other groups. It has been reported that an MGL of >32\% is likely to be accompanied with associated detectable clinical symptoms.\textsuperscript{[10]} Similarly, several studies have found correlation between MGL and OSDI scores.\textsuperscript{[25,36]} On the other hand, other studies have not found any correlation between MGL and OSDI scores \textsuperscript{[26,35]} or SPEED questionnaire. \textsuperscript{[37]}

Regarding MGL and its correlation with other clinical parameters, statistically significant differences were found in TFO between group 5 and groups 1,2 and 3. Besides, statistically significant differences were found in corneal staining as well as conjunctival staining between group 5 and groups 1 and 2. These results are in agreement with those obtained by Feng et al. \textsuperscript{[26]} who found a positive correlation between corneal staining and MGL in DED patients. Previous studies have suggested that when the amount of MG reduced, the secretion of MG decreases. This might induce tear film homeostasis loss and greater tear film evaporation which could lead to surface epithelial damage, and disturbance of the glycocalyx and goblet cell mucins.\textsuperscript{[38]}

In the present study no differences were found in TBUT and Schirmer test among different MGL groups. These results are in accordance with other studies that did not find any correlation between MGL and these tear film parameters \textsuperscript{[39]} or a
low correlation was found.[26] Nevertheless, Arita et al. [40] studied a population
with MGD and found that Schirmer test was positively correlated with the
meiboscore. Thus, patients with less amount or damaged MG would have an
increase of fluid that may compensate the decreased function of the lipid layer.
All these findings together suggest that these ocular surface parameters could
be affected by MGL in patients who suffer from MGD since the glandular loss
may exacerbate the signs of MGD in comparison with those who only present
MGL without other ocular condition.
No statistically significant differences were found in any of the 5KM parameters
among MGL groups except for BR. Recently, Ji et al. [41] found a correlation
between MGL grade and NIKBUT-avg, NIKBUT and LLT in patients with DED
and MGD. This discrepancy between studies could due to the population studied.
Indeed, the correlation found might be because they only included subjects with
DED and MGD.
Our findings suggest that a MGL higher than 50% is accompanied by signs of
increased osmolarity, redness and staining of the ocular surface. However, it is
important to highlight that the mean age of the participants in this study was
higher in those groups with higher MGL. Therefore, it is not possible to ascertain
if these signs in ocular surface parameters are due to MGL or to age. Indeed, the
great influence of aging on MG morphology and function is well-known and
documented in the literature [7,18,27,39]. In order to address this point, the
relationship between MGL and the ocular surface parameters was assessed
considering age as a covariant. When it was performed, only the corneal staining
was correlated with MGL. These results emphasize the influence of ageing in the
MG morphology but also in several ocular surface parameters such as corneal
staining, LLT or tear volume among others. [27,42] This has been previously reported [27] and points out the importance of considering participants age when performing research studies focused on the ocular surface. Thus, in order to assess the real impact or influence of the MGL in ocular surface parameters (which are influenced by age), it would be necessary to compare matched age groups. For example, MGL has been found to be positively correlated with meibum quality suggesting an impaired MG function when MGL increase.[19,41,43] The present study has shown that when age is covariant, the relationship between MGL and meibum quality is absent, indicating that meibum quality could be decreased either because a higher amount of MGL or because it is naturally decreased with aging. These findings suggest that different thresholds for defining abnormal ocular and tear film surface parameters should be considered according with the age. Next steps should be focused on determining the normal values of different parameters for each age range.

In summary, this study suggests that a MGL higher than 50% is accompanied by signs in the ocular surface. As well, in the light of the findings, future studies must consider age due to its great influence on the MG morphology and compare matched-age groups in order to know the contribution of the MGL on the ocular surface as well as establish valid cut-off values for DED diagnosis.
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The authors have no proprietary interest in any of the devices mentioned in this article.
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B. Risk Factors and Symptoms of Meibomian Gland Loss in a Healthy


FIGURES LEGENDS

Figure 1. Flow diagram of the study protocol. Numbers indicate the order of performance of each test (from less invasive towards to the most invasive).

Figure 2. Different grades of MGL in the UL and LL obtained by non-contact infrared meibography (Oculus Keratograph K5M). A.1 and A.2) Participants without MGL in the UL and LL (total meiboscore 0; = 0%). B.1 and B.2) Participants with slight MGL in the UL and LL (total meiboscore 1; < 33%). C.1 and C.2) Participants with moderate MGL in the UL and LL (total meiboscore 2; 33-66%). D.1 and D.2) Participant with severe MGL in the UL and LL (total meiboscore 3; >66%).
Clinical Protocol

1. Subjective Symptomatology
   - Tear Film Osmolarity
     - General Examination
       - Cornea
         - Fluorescein dye
         - TBUT
         - Corneal staining
       - Conjunctiva
         - Lissamine green dye
         - Conjunctival staining
         - Lid wiper Epitheliopathy
     - Upper and Lower Meibomian gland loss

2. Tear Film Stability
   - Keratograph SM
     - Tear Film Meniscus
     - Ocular Hyperemia
     - Tear film stability

3. Slit lamp Assessment

4. Meibography

5. Tear Film Volume
   - Schirmer test with topical anaesthesia
**Table 1.** Demographic data from the participants of the study. Results are expressed in mean± standard deviation (SD) for each parameter.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td><strong>N</strong></td>
<td>161</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>42±17</td>
</tr>
<tr>
<td><strong>Age range (years)</strong></td>
<td>19 to 88</td>
</tr>
<tr>
<td><strong>Male/ Female (%)</strong></td>
<td>43/57</td>
</tr>
<tr>
<td><strong>OSDI (scores)</strong></td>
<td>17.47±15.65</td>
</tr>
<tr>
<td><strong>SPEED (scores)</strong></td>
<td>7±5</td>
</tr>
<tr>
<td><strong>TFO (mOsms/L)</strong></td>
<td>309±17</td>
</tr>
<tr>
<td><strong>TMHk (mm)</strong></td>
<td>0.26±0.08</td>
</tr>
<tr>
<td><strong>REDNESS (scores)</strong></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Limbal</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Bulbar</strong></td>
</tr>
<tr>
<td><strong>NIKBUT (seconds)</strong></td>
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</tr>
<tr>
<td></td>
<td><strong>First</strong></td>
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<td><strong>Average</strong></td>
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<td>4.36±2.07</td>
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<tr>
<td><strong>SCHIRMER TEST (mm)</strong></td>
<td>11.83±7.14</td>
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</tbody>
</table>

OSDI: The Ocular-surface-disease-index; SPEED: Standard Patient Evaluation of Eye Dryness; TFO: tear film osmolarity; TMHk: tear meniscus height; Bulbar and limbal redness were graded automatically by K5M software; NIKBUT-FR: first rupture non-invasive Keratograph tear film break-up time; NIKBUT-AVG: average of non-invasive Keratograph tear film break-up time.
Table 2. Contingency table of MGL for UL and LL.

<table>
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<tr>
<th>Lower Eyelid</th>
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<td>4</td>
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<td></td>
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<td>0%</td>
<td>36.84%</td>
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</tr>
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<td>1</td>
<td>33</td>
<td>36</td>
<td>12</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>39.76%</td>
<td>43.37%</td>
<td>14.46%</td>
<td>2.41%</td>
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<td>3</td>
<td>8</td>
<td>9</td>
<td>3</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.04%</td>
<td>34.78%</td>
<td>39.13%</td>
<td>13.04%</td>
<td>5.26%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>33.33%</td>
<td>0%</td>
<td>66.67%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

| Column Totals | 57 | 67 | 25 | 7 | 156 |

UL: Upper eyelid; LL: Lower eyelid; MGL: Meibomian gland loss
*Meiboscore: Grade 0, no gland loss; grade 1, area of gland loss <33% of the total gland area; grade 2, area of gland loss 33%–67%; and grade 3, area of gland loss >67%
Table 3. Characteristics and distribution of the sample according the MGL grade (meiboscore). Results expressed in mean± standard deviation (SD) and percentage (%).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Meiboscore</strong>&lt;sup&gt;*&lt;/sup&gt; (reference)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4+5+6</td>
</tr>
<tr>
<td><strong>MGL (%)</strong></td>
<td>0</td>
<td>0-16.5</td>
<td>16.5-33</td>
<td>33-49.5</td>
<td>&gt;50</td>
</tr>
<tr>
<td><strong>N (%)</strong></td>
<td>21 (13.04%)</td>
<td>59 (36.65%)</td>
<td>44 (27.33%)</td>
<td>20 (12.42%)</td>
<td>17 (10.55%)</td>
</tr>
<tr>
<td><strong>AGE (years)</strong></td>
<td>34±11</td>
<td>37±15</td>
<td>46±17</td>
<td>50±18</td>
<td>60±18</td>
</tr>
<tr>
<td><strong>FEMALE (%)</strong></td>
<td>9/91 9.9%</td>
<td>33/91 36.3%</td>
<td>27/91 29.7%</td>
<td>8/91 8.8%</td>
<td>12/91 13.2%</td>
</tr>
<tr>
<td><strong>MALE (%)</strong></td>
<td>12/70 17.1%</td>
<td>25/70 35.7%</td>
<td>17/70 24.3%</td>
<td>12/70 17.1%</td>
<td>4/70  5.7%</td>
</tr>
</tbody>
</table>

<sup>*</sup>The total meiboscore is the sum of the meiboscore of both eyelids (0-6). MGL: Meibomian gland loss.
Table 4. Comparison of symptomatology scores among different MGL grades. Results expressed in mean± standard deviation (SD) for each parameter (units).

<table>
<thead>
<tr>
<th>TOTAL MEIBOSCORE GROUP (MGL%)</th>
<th>SYMPTOMATOLOGY</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OSDI</td>
<td>SPEED</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(scores)</td>
<td>(scores)</td>
</tr>
<tr>
<td>Group 1 (0)</td>
<td>14.30±13.95</td>
<td>7±5</td>
<td></td>
</tr>
<tr>
<td>Group 2 (0-16.5)</td>
<td>16.34±13.90</td>
<td>7±5</td>
<td></td>
</tr>
<tr>
<td>Group 3 (16.5-33)</td>
<td>18.30±16.76</td>
<td>7±5</td>
<td></td>
</tr>
<tr>
<td>Group 4 (33-49.5)</td>
<td>15.61±15.21</td>
<td>6±5</td>
<td></td>
</tr>
<tr>
<td>Group 5 (&gt;50)</td>
<td>23.28±23.28</td>
<td>9±5</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.385</td>
<td>0.506</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant differences among groups; p<0.05.
Table 5. Comparison of classical clinical parameters among different MGL grades. Results expressed in mean± standard deviation (SD) for each parameter (units).

<table>
<thead>
<tr>
<th>TOTAL MEIBOSCORE GROUP (MGL%)</th>
<th>CLASSICAL CLINICAL PARAMETERS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TFO (mOsms/L)</td>
<td>TBUT (seconds)</td>
</tr>
<tr>
<td>Group 1 (0)</td>
<td>308±16</td>
<td>4.86±2.52</td>
</tr>
<tr>
<td>Group 2 (0-16.5)</td>
<td>309±19</td>
<td>4.85±2.57</td>
</tr>
<tr>
<td>Group 3 (16.5-33)</td>
<td>304±15</td>
<td>4.04±1.46</td>
</tr>
<tr>
<td>Group 4 (33-49.5)</td>
<td>312±12</td>
<td>3.96±1.22</td>
</tr>
<tr>
<td>Group 5 (&gt;50)</td>
<td>326±18</td>
<td>3.64±1.51</td>
</tr>
<tr>
<td>p-value</td>
<td>0.023*</td>
<td>0.249</td>
</tr>
</tbody>
</table>

* statistically significant differences among groups; p<0.05.
TFO: tear film osmolarity; TBUT: tear break-up time; Corneal and conjunctival staining were graded using Oxford Staining Score System.
Table 6. Comparison of K5M automated measurements among different MGL grades. Results expressed in mean± standard deviation (SD) for each parameter (units).

<table>
<thead>
<tr>
<th>TOTAL MEIBOSCORE GROUP (MGL%)</th>
<th>K5M AUTOMATED MEASUREMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMHk (mm)</td>
</tr>
<tr>
<td>Group 1 (0)</td>
<td>0.23±0.05</td>
</tr>
<tr>
<td>Group 2 (0-16.5)</td>
<td>0.26±0.71</td>
</tr>
<tr>
<td>Group 3 (16.5-33)</td>
<td>0.27±0.89</td>
</tr>
<tr>
<td>Group 4 (33-49.5)</td>
<td>0.26±0.80</td>
</tr>
<tr>
<td>Group 5 (&gt;50)</td>
<td>0.28±0.96</td>
</tr>
<tr>
<td>p</td>
<td>0.405</td>
</tr>
</tbody>
</table>

*p statistically significant differences among groups; p<0.05.

TMHk: tear meniscus height; Bulbar (BR) and limbal redness (LR) were graded automatically by K5M software; NIKBUT-FR: first rupture non-invasive Keratograph tear film break-up time; NIKBUT-AVG: average of non-invasive Keratograph tear film break-up time.
Table 7. Relationship between MGL and the ocular surface parameters considering age as covariant.

<table>
<thead>
<tr>
<th></th>
<th>MGL (Covariant: Age)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td></td>
</tr>
<tr>
<td><strong>DED Questionnaires</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSDI</td>
<td>0.011</td>
<td>0.914</td>
</tr>
<tr>
<td>SPEED</td>
<td>0.012</td>
<td>0.904</td>
</tr>
<tr>
<td><strong>K5M Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMHk</td>
<td>0.059</td>
<td>0.563</td>
</tr>
<tr>
<td>BR</td>
<td>-0.003</td>
<td>0.969</td>
</tr>
<tr>
<td>LR</td>
<td>-0.044</td>
<td>0.668</td>
</tr>
<tr>
<td>NIKBUT-fr</td>
<td>-0.095</td>
<td>0.345</td>
</tr>
<tr>
<td>NIKBUT-avg</td>
<td>-0.054</td>
<td>0.597</td>
</tr>
<tr>
<td><strong>Classical Clinical Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFO</td>
<td>0.088</td>
<td>0.393</td>
</tr>
<tr>
<td>Corneal staining</td>
<td>0.208</td>
<td>0.041*</td>
</tr>
<tr>
<td>Conjunctival staining</td>
<td>-0.004</td>
<td>0.966</td>
</tr>
<tr>
<td>TBUT</td>
<td>-0.163</td>
<td>0.112</td>
</tr>
<tr>
<td>Schirmer test</td>
<td>-0.065</td>
<td>0.527</td>
</tr>
<tr>
<td><strong>MG features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG quality secretion</td>
<td>0.044</td>
<td>0.587</td>
</tr>
</tbody>
</table>

*statistically significant; p<0.05.
(r, Spearman correlation coefficient)

OSDI: The Ocular-surface-disease-index (scores); SPEED: Standard Patient Evaluation of Eye Dryness (scores); TMHk: tear meniscus height (mm); Bulbar and limbal redness (BR and LR) were graded automatically by K5M software; NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); TFO: tear film osmolarity(mOsms/L); TBUT: tear break-up time (seconds); Schirmer test (mm); Corneal and conjunctival staining were graded using Oxford Staining Score System; MG: Meibomian glands