

# Meibomian Gland Morphology Is a Sensitive Early Indicator of Meibomian Gland Dysfunction



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- **PURPOSE:** To investigate the relationship between meibomian gland (MG) morphology and clinical dry eye tests in patients with meibomian gland dysfunction (MGD).
- **DESIGN:** Cross-sectional study.
- **SUBJECTS:** Total 538 MGD patients and 21 healthy controls.
- **METHODS:** MG loss on meibography images of upper (UL) and lower lids (LL) was graded on a scale of 0 (lowest degree of MG loss) to 3. MG length, thickness, and interglandular space in the UL were measured. Clinical tests included meibum expression and quality, tear film break-up time, ocular staining, osmolarity, Schirmer I, blink interval timing, and Ocular Surface Disease Index (OSDI) questionnaire.
- **RESULTS:** Mean UL and LL meibogrades were significantly higher in MGD patients compared to controls ( $P < .001$  for UL and LL). The sensitivity and specificity of the meibograde as a diagnostic parameter for MGD was 96.7% and 85%, respectively. Schirmer I was significantly increased in MGD patients with meibograde 1 compared to patients with meibograde 0, 2, and 3 in the UL ( $P < .05$ ). MG thickness increased with higher meibograde ( $P < .001$ ). MG morphology correlated significantly but weakly with several clinical parameters ( $P < .05$ ). OSDI did not correlate with any MG morphologic parameter.

- **CONCLUSIONS:** Grading of MG loss using meibograde effectively diagnoses MGD. Compensatory mechanisms such as increased aqueous tear production and dilation of MGs make early detection of MGD difficult by standard clinical measures of dry eye, whereas morphologic analysis of MGs reveals an early stage of MGD, and therefore represents a complementary clinical parameter with diagnostic potential. (Am J Ophthalmol 2019;200:16–25. © 2018 Elsevier Inc. All rights reserved.)

**M**EIBOMIAN GLANDS (MG) ARE RESPONSIBLE FOR the secretion of meibum, which constitutes the main lipid component of the outer layer of the tear film.<sup>1</sup> These lipids stabilize and prevent early evaporation of the tear film, and are consequently critical to ensure a healthy ocular surface. Therefore, alterations in meibum, whether owing to deficiencies in secretion or to the composition of lipids, have adverse effects on the tear film, resulting in evaporative dry eye disease (DED).<sup>1–3</sup> Meibomian gland dysfunction (MGD) is reported to be the most common cause of evaporative DED, and is a frequent condition encountered in ophthalmologic practice.<sup>4–7</sup> Epidemiologic studies report the prevalence of MGD in different populations to range from 38% to 68%.<sup>8</sup>

Recent advances in noninvasive techniques of visualizing MG morphology provide clinicians with a novel tool in diagnosis and classification of MGD.<sup>9–11</sup> Nevertheless, studies investigating the relationship between MG morphology and clinical tests associated with DED report inconsistent results.<sup>12–17</sup> Previous reports also showed that certain morphologic characteristics such as MG thickness, length, and tortuosity are associated with different stages of MGD.<sup>1,2,18</sup> The nature of MGD, with its various presentations, has therefore resulted in a lack of clearly defined diagnostic criteria and an absence of effective diagnostic tools for MGD.

Loss of MG tissue is most commonly the sole investigated characteristic on meibography images, and the International Workshop on MGD recognizes MG loss as a key clinical sign of MGD.<sup>1,9,19–21</sup> Studies have, however, shown a regional difference of actively secreting MGs on the tarsal plate,<sup>22</sup> compensatory thickening in early-stage MGD,<sup>18,23</sup> and a low utilization of the meibum reservoir

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in healthy subjects.<sup>24,25</sup> Therefore, morphologic alterations in MGD should not be restricted to atrophy alone. Further analyses of additional parameters of MG morphology and function and their relation to clinical dry eye tests are required.

In this study, we aimed to evaluate MG morphology as a potential discriminator between MGD patients and healthy subjects, and to better understand the pathophysiological changes that occur with MGD development. Herein, we investigated the relationship between morphologic characteristics of MGs visualized by meibography and clinical dry eye tests, in a large sample of a Norwegian cohort of patients with MGD. Additionally, we explored the differences in clinical and morphologic parameters in distinct MG loss groups, compared with age-matched healthy subjects.

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## METHODS

A TOTAL OF 538 PATIENTS SEEKING CONSULTATION AT THE Norwegian Dry Eye Clinic, and diagnosed with MGD according to the guidelines from 2011,<sup>1</sup> were included in this cross-sectional study. Briefly, the diagnosis of MGD is made after first diagnosing evaporative DED, based on symptoms assessment and tear film break-up time (TFBUT), and supplementary clinical tests such as blink rate and interval timing, tear film osmolarity measurement, Schirmer I test, and ocular staining. Additionally, MGD-specific assessments of morphologic eyelid features, meibum expressibility and quality, and evaluation of gland dropout on meibography images are used to separate MGD from other subtypes of DED. All data were collected from the first consultation at the clinic. Twenty-one healthy volunteers, with no preexisting ocular or systemic conditions or symptoms of DED, were recruited through the National Centre for Optics, Vision and Eye Care as a control group. Written informed consent was obtained from all participants prior to examination. The study was conducted in accordance with the Declaration of Helsinki. The Regional Committee for Medical & Health Research Ethics, Section C, South East Norway (REC) reviewed the use of the data material from the clinic. REC found the research project “*Evaluation of data from the Norwegian Dry Eye Clinic*” to be outside the remit of the Act on Medical and Health Research (2008) and, therefore, able to be implemented without specific approval. A letter of exemption from REC has been provided.

• **MEIBOGRAPHY:** Meibography images were acquired by the noncontact infrared meibography system Oculus Keratograph 5 (Oculus, Wezlar, Germany). Images of both upper (UL) and lower (LL) eyelids of both eyes were analyzed. Subjects were excluded on the basis of unsatisfactory meibography images by the application of the following exclusion criteria: (1) interrupted complete assessment of

eyelid (ie, nail/finger); (2) inadequate exposure of the tarsal area; (3) interfering reflection; or (4) lack of focus/blurred image.

An experienced observer subjectively evaluated the MG loss in both UL and LL using a validated meibograde grading scheme, with a 4-point scale from 0 to 3, where grade 0 = area of MG loss between 0 and 25%; grade 1 = area of MG loss between 26% and 50%; grade 2 = area of MG loss between 51% and 75%; and grade 3 = area of MG loss 76%-100% (Figure 1). The area of MG loss was evaluated in reference to expected normal MG area in healthy subjects, equivalent to the tarsal plate.<sup>10</sup>

Following the subjective evaluation, an extensive computerized objective assessment was performed with ImageJ (v. 2.0.0; National Institutes of Health, Bethesda, Maryland, USA) software. Briefly, the tarsal area was outlined, as the assumed normal MG area, using ImageJ's polygon function. The outer boundaries of this area were identified according to the definitions proposed by Pult and associates.<sup>19</sup> To increase visibility of the MGs, local contrast was enhanced using a plug-in function in ImageJ. Finally, the actual MG area was outlined, and the percentage of MG loss was calculated by dividing the MG area by the tarsal area (Figure 2A and B).

Additional computerized analyses of MG morphology included measurements of length, thickness, and interglandular space (ie, the space between 2 adjacent MGs), which were performed on the 3 most representative glands in the UL only, as it often was difficult to distinguish separate MGs in the LL for morphologic investigations (Figure 2C). Average values of these measurements were used for further statistical analysis. Furthermore, the number of tortuous MGs (tortuosity) was reported, with a tortuous gland being defined as having at least 1 angle greater than 45 degrees.

• **CLINICAL DRY EYE TESTS:** Patients first completed a symptom questionnaire to give an Ocular Surface Disease Index (OSDI) score between 0 (no symptoms) and 100 (severe symptoms).<sup>26</sup> Meibum expression was measured by application of firm digital pressure to the central area of the LL to observe the number of active MGs. Five MGs in the central area were tested for their ability to express meibum. This result was scored from 0 to 3: 0 = all glands expressible; 1 = 3-4 glands expressible; 2 = 1-2 glands expressible; and 3 = no glands expressible.<sup>27</sup> Meibum quality of the central 8 MGs was scored from 0 to 3: 0 = clear fluid; 1 = cloudy fluid; 2 = cloudy, particulate fluid; and 3 = opaque, toothpaste-like meibum.<sup>28</sup> The score for each gland was added to give a total score. MGs that were not able to express meibum were scored 0 for meibum quality with an additional note in the journal system.<sup>1</sup>

Ocular staining and TFBUT were measured 0.5 minutes after instillation of 5  $\mu$ L 0.5% fluorescein to the conjunctival sac of each eye, and graded according to the Oxford grading system.<sup>1,29</sup> Furthermore, to quantitatively assess

# Meibograde

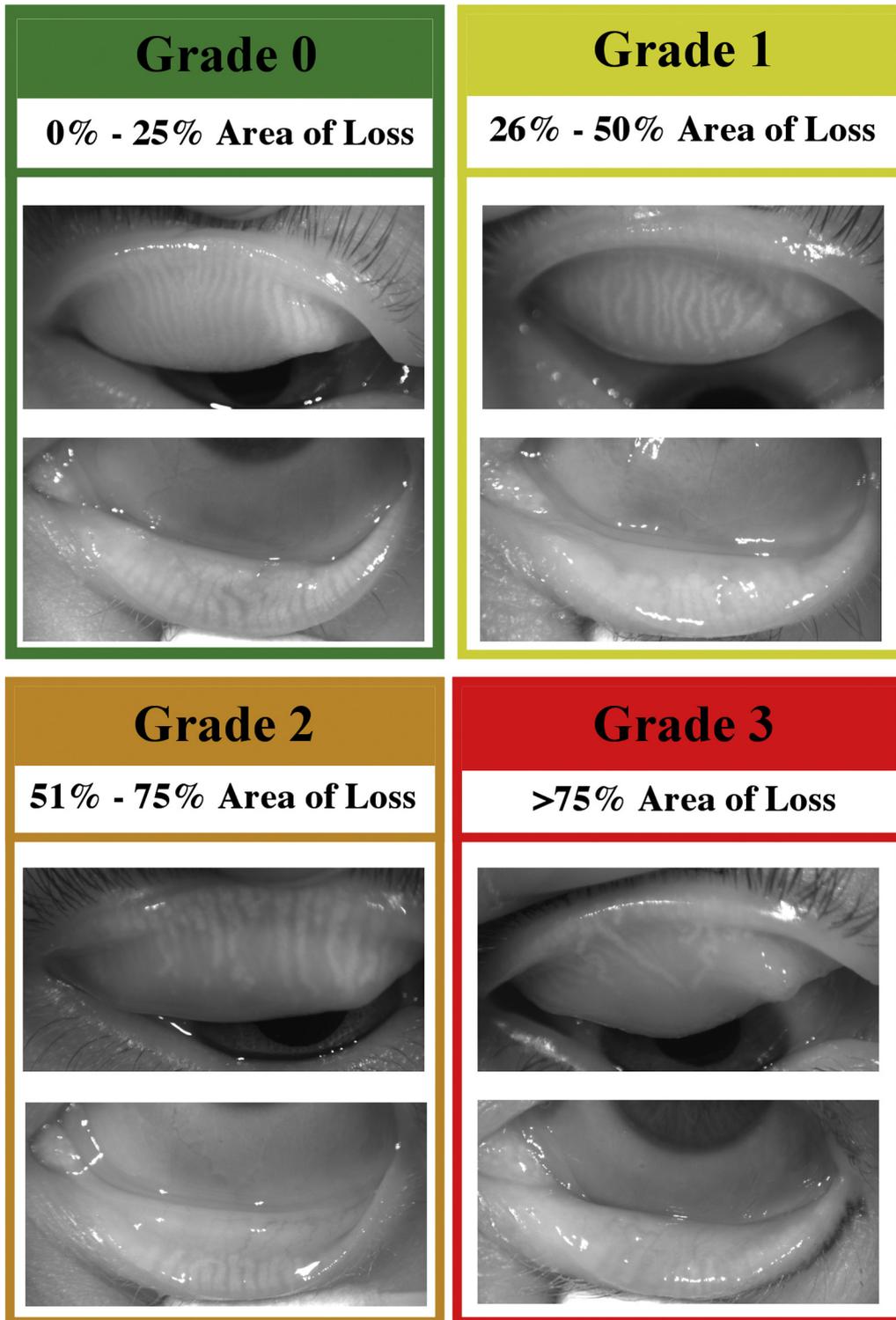


FIGURE 1. The Meibograde grading system: subjective grading of meibomian gland loss.

tear secretion, Schirmer I test was performed without anesthesia by inserting the test strip in the lateral third of the lower eyelid for 5 minutes.<sup>1</sup>

Additional clinical tests included timing of blink interval, measurement of tear film osmolarity with the TearLab TM system (TearLab Corporation, San Diego, California,

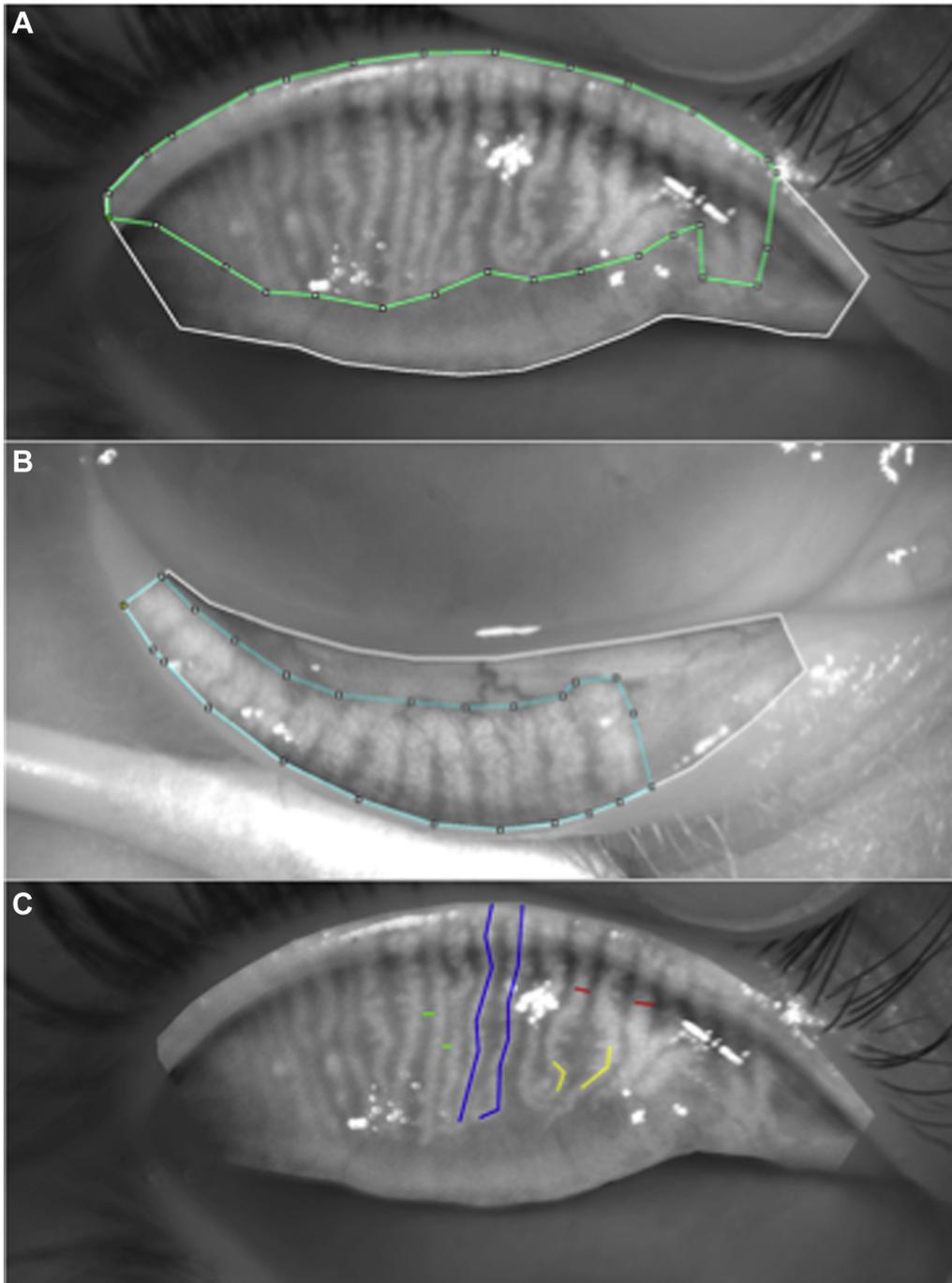


FIGURE 2. Computerized grading of meibomian gland loss in the upper eyelid (A) and lower eyelid (B) using ImageJ software. The contrast is enhanced on the tarsal area to improve visualization of the meibomian glands. (C) Computerized semi-objective measurements of meibomian gland length (blue), thickness (red), interglandular space (green), and tortuosity (yellow).

USA), and evaluation of dry eye severity level (DESL) from 1 to 4 according to the guidelines proposed by the 2007 International Dry Eye Workshop.<sup>30</sup>

• **STATISTICAL ANALYSIS:** Statistical analysis was performed using SPSS (v. 24.0, IBM Corp., Armonk,

New York, USA) and GraphPad Prism (v.7.0, GraphPad Software, San Diego, California, USA) statistical software. Data were tested for normal distribution by Shapiro-Wilk test. Nonparametric tests were used; Spearman rank correlation coefficient was calculated for correlations, Mann-Whitney *U* statistics and Kruskal-Wallis with Dunn's

**TABLE 1.** Age and Number of Meibomian Gland Dysfunction Patients With Different Meibogrades in the Upper and Lower Eyelid

Meibograde	0	1	2	3	P Value
UL	49.2 ± 16 (n = 352)	50.1 ± 16.6 (n = 275)	51 ± 17.2 (n = 185)	51.4 ± 15.9 (n = 158)	.758
LL	50.2 ± 17.1 (n = 101)	50 ± 15.7 (n = 250)	49.6 ± 16.4 (n = 363)	51 ± 16.7 (n = 256)	.441

LL = lower lid; UL = upper lid.

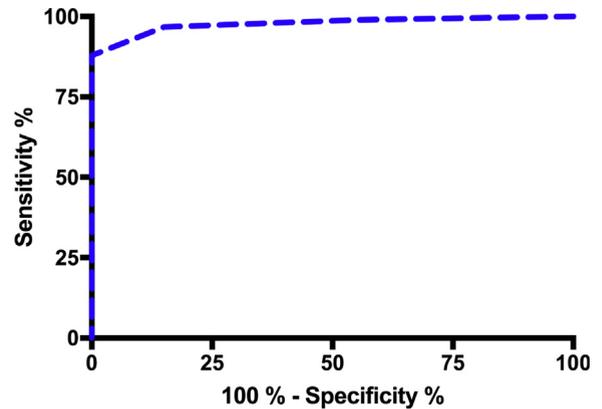
The subgroups of meibomian gland dysfunction patients with different meibogrades are age-matched. All results are represented in years and n is the number of eyelids.

post hoc test were performed to compare groups. For comparisons between the patient group and the control group, the influence of age was adjusted for using a general linear model (GLM). The significances of multiple correlations were corrected with the 2-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli (q-value was set at 1%). A receiver operating characteristic (ROC) curve was generated to investigate the clinical application and cut-off values of the meibograde system for establishing MGD diagnosis. With the exception of ROC statistics, values from the right and left eyes were combined to give one value for UL and one for LL in all subjects. For ROC statistics, all 4 eyelids were averaged to give one meibograde for each subject. Clinical dry eye tests that were performed on each eye separately were combined to give a mean value. A *P* value < .05 was considered significant.

## RESULTS

FIFTY-ONE PATIENTS AND ONE CONTROL SUBJECT WERE excluded from the study prior to commencing analyses owing to unsatisfactory meibography images according to the exclusion criteria. Thus, meibography images of 487 patients (361 female and 126 male; age: 50.13 ± 16.37 years, range: 9-88 years) and 20 healthy controls (12 female and 8 male; age: 31.7 ± 14 years, range: 19-65 years) were included, resulting in a total of 2028 images.

We found significantly higher values in mean meibograde, as well as percentage dropout by computerized assessment, for both UL and LL in MGD patients compared to that of healthy subjects. Mean UL and LL meibogrades were 1.15 ± 1.09 and 1.8 ± 0.95, respectively, in patients, while these values were 0.1 ± 0.3 and 0.25 ± 0.49 in the control group (*P* < .001 for controls vs patients, for UL and LL). The distribution of MGD patients with different meibogrades can be seen in Table 1. Using computerized analysis, the mean percentage MG loss in the UL was 31.3% ± 17% in patients and 13.8% ± 5.5% in controls, while dropout in the LL was 51.7% ± 14.6% in patients and 23.6% ± 13.2% in controls (*P* < .001 for controls vs patients, for UL and LL). These data suggested increased loss of MG tissue in MGD patients compared to healthy subjects.



**FIGURE 3.** Receiver operating characteristic curve demonstrating the diagnostic ability of meibogrades for diagnosing meibomian gland dysfunction (MGD). Our results demonstrated excellent ability of the subjective meibograde to distinguish between MGD patients and healthy controls. Using an average meibograde of all 4 eyelids in a subject, a cut-off value of average meibograde 0.5 for MGD resulted in a sensitivity and specificity of 96.7% and 85%, respectively.

We generated an ROC curve and calculated the area under the curve (AUC) to assess the ability of the subjective meibograde grading system to discriminate between MGD patients and healthy controls (Figure 3). The highest AUC, and consequently the optimal cut-off value, was achieved using an average of meibogrades for ULs and LLs in both the right and left eyes (average meibograde of all 4 eyelids). The average meibograde for patients was significantly higher than control values (mean 2.57 ± 1.32 and 0.35 ± 0.37, respectively, Mann-Whitney *U*, *P* < .001). The AUC was calculated to be 0.970 (*P* < .001). For a cut-off value of average meibograde 0.5, the sensitivity and specificity were 96.7% and 85%, respectively. Using average meibograde 0.75 as a cut-off value resulted in a sensitivity of 87.9% and a specificity of 100%. Furthermore, 481 out of 487 MGD patients had an average meibograde of at least 0.5. These numbers demonstrate that the symptomatic MGD population in our study with average meibograde below 0.5 is quite sparse.

Schirmer I test was significantly higher in patients with meibograde 1 in the UL compared to patients with

**TABLE 2.** Comparison of Clinical Test Results Between Meibomian Gland Dysfunction Patients With Different Meibogrades in the Upper and Lower Eyelids

Test	Meibograde				Kruskal-Wallis <i>P</i> Value
	0	1	2	3	
<b>Upper Lid</b>					
OSDI	33.6 ± 22.7	37.6 ± 23.7	38.8 ± 20.3	33 ± 21.6	<.05
DESL	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.4	2.2 ± 0.4*	<.001
Osmolarity (mOsmol/L)	311.8 ± 14.1	312.5 ± 15.9	313.1 ± 17.2	310.4 ± 14.4	.793
TFBUT (s)	4.2 ± 3	3.8 ± 2.8	3.2 ± 2.4*	3.3 ± 2.1	.001
Blink interval (s)	3.3 ± 2.7	3.7 ± 4.4	3.5 ± 5.1	2.7 ± 1.6	.061
Schirmer I (mm)	14.7 ± 9.6	16.9 ± 9.3*	14.2 ± 9.5	14.2 ± 9.4	.001
Ocular staining	1.4 ± 1.8	1.6 ± 2.2	1.6 ± 2	1.9 ± 2.1*	.004
Meibum expression	0.9 ± 0.9	1.2 ± 0.9*	1.4 ± 0.9*	1.7 ± 0.9*	<.001
Meibum quality	9.4 ± 4.5	10.3 ± 4.5	8.6 ± 4.8	9.2 ± 6	.003
<b>Lower Lid</b>					
OSDI	40.3 ± 23.4	36.2 ± 22.5	33.9 ± 22.2	35.9 ± 22.6	.133
DESL	2 ± 0.2	2.1 ± 0.3	2.1 ± 0.3	2.2 ± 0.4*	.004
Osmolarity (mOsmol/L)	311.9 ± 13.9	312.4 ± 16.2	312.5 ± 15	311 ± 15.2	.8
TFBUT (s)	3.8 ± 2.6	3.7 ± 2.7	3.8 ± 2.8	3.9 ± 2.7	.678
Blink interval (s)	3.2 ± 2.4	3.1 ± 2.1	3.8 ± 5.3	3.2 ± 2.3	.183
Schirmer I (mm)	15.6 ± 10.1	14.4 ± 9.5	15.2 ± 9.8	14.3 ± 9.2	.568
Ocular staining	1 ± 1.2	1.5 ± 2	1.7 ± 2.1	1.7 ± 2*	.022
Meibum expression	1.1 ± 0.9	1.1 ± 0.9	1.1 ± 0.9	1.5 ± 1*	<.001
Meibum quality	9.1 ± 4	10 ± 4.6	9.3 ± 4.4	9.3 ± 5.8	.263

DESL = dry eye severity level; LL = lower lid; OSDI = Ocular Surface Disease Index; TFBUT = tear film break-up time; UL = upper lid. Values indicated by asterisk (\*) are significant compared to meibograde 0.

**TABLE 3.** Comparison of Clinical Dry Eye Test Results Between Meibomian Gland Dysfunction Patients and Healthy Controls

	Patients	Controls	Mann-Whitney <i>U</i> <i>P</i> Value
Osmolarity (mOsmol/L)	311.9 ± 14.4	305 ± 10.5	.006
TFBUT (s)	3.7 ± 2.7	9.6 ± 10	<.001*
Blink interval (s)	3.4 ± 4.1	4.3 ± 3.9	.01*
Schirmer 1 (mm)	14.4 ± 10	16.5 ± 11.9	.4
Ocular staining	1.6 ± 2	1 ± 0.7	.673
Meibum expression	1.2 ± 0.9	0.8 ± 0.8	.002
Meibum quality	9.3 ± 4.9	4 ± 0.2	<.001*

TFBUT = tear film break-up time.

*P* values designated with an asterisk (\*) indicate significance after adjusting for the influence of age using a general linear model.

meibograde 0, 2, and 3 ( $P < .05$ ) (Table 2). Additionally, the mean value of the Schirmer I test among all MGD patients was insignificant from the control group,  $14.4 \pm 10$  mm in patients and  $16.5 \pm 11.9$  mm in the control group ( $P = .40$ ) (Table 3).

MG thickness in the UL increased significantly with higher subjective meibograde in the MGD group (Kruskal-Wallis  $P < .001$ ). Interglandular space increased with progressive MG loss despite increasing MG thickness, whereas MG length and tortuosity was extensively reduced with higher meibograde ( $P < .001$ ) (Table 4).

We found several changes in clinical dry eye tests in both ULs and LLs with different meibogrades in the MGD group (Table 2). Ocular staining, DESL, and meibum expression were increased in patients with higher meibogrades, whereas changes in OSDI, TFBUT, and meibum quality in the UL were significant, but subtle, and did not follow any particular pattern. Osmolarity was significantly increased in MGD patients in comparison to controls (Table 3).

Among MGD patients, meibum expression correlated significantly with percentage MG loss (by computerized assessment) in the UL ( $r = 0.368$ ,  $P < .001$ ) and LL

**TABLE 4.** Comparison of Morphologic Parameters Between Meibomian Gland Dysfunction Patients With Different Meibogrades in the Upper Eyelid

Parameter <sup>a</sup>	UL Meibograde				Kruskal-Wallis P Value
	0	1	2	3	
Thickness	18.8 ± 3.3	19.4 ± 3.6	19.9 ± 3.7*	20.6 ± 4.5*	<.001
Interglandular space	14.9 ± 2.7	15.4 ± 3	15.5 ± 3.2	16.8 ± 3.6*	<.001
Length	308.2 ± 53.4	288.5 ± 52.5*	219.5 ± 50.2*	147 ± 53.2*	<.001
Tortuosity	1.3 ± 1.4	1.4 ± 1.4	0.7 ± 1.2*	0.2 ± 0.6*	<.001

Values indicated by an asterisk (\*) are significant compared to meibograde 0.  
Values are given in ImageJ pixels.  
<sup>a</sup>Thickness, interglandular space, length, and tortuosity all refer to meibomian glands.

**TABLE 5.** Correlation Coefficients of Clinical Dry Eye Tests and Meibomian Gland Morphology in Meibomian Gland Dysfunction Patients

	OSDI	DESL	TFBUT	Osmolarity	Blink Interval	Schirmer I	Ocular Staining	Meibum Expression	Meibum Quality
UL meibograde	0.031	0.151*	-0.122*	-0.025	-0.069*	-0.025	0.100*	0.290*	-0.064
LL meibograde	-0.045	0.110*	0.031	-0.023	0.020	-0.011	0.090*	0.132*	-0.048
UL % MG loss	0.016	0.151*	-0.095*	0.011	-0.079	-0.031	0.110*	0.368*	-0.075
LL % MG loss	-0.038	0.151*	0.037	0.017	0.034	0.016	0.135*	0.202*	-0.078
Thickness	-0.006	0.006	-0.027	-0.196*	-0.043	-0.057	-0.016	-0.129*	0.002
Interglandular space	-0.019	0.027	-0.058	-0.138*	-0.045	0.014	0.011	-0.104*	0.043
Length	-0.010	-0.142*	0.084	-0.040	0.099*	0.042	-0.126*	-0.286*	0.076
Tortuosity	-0.065	-0.056	0.017	-0.020	0.077	0.044	-0.039	-0.107*	0.049

DESL = dry eye severity level; LL = lower lid; MG = meibomian gland; OSDI = Ocular Surface Disease Index; TFBUT = tear film break-up time; UL = upper lid.

Significant values are indicated by an asterisk (\*) and were corrected for false discoveries using the procedure of Benjamini, Krieger, and Yekutieli (q-value was set at 1%).

( $r = 0.202$ ,  $P < .001$ ), and inversely to MG length ( $r = -0.286$ ,  $P < .001$ ). MG thickness was inversely correlated to osmolarity ( $r = -0.198$ ,  $P < .001$ ). Weak but significant correlations were found for several parameters (Table 5). OSDI did not correlate with any parameter of MG morphology. Age was not correlated with either UL meibograde ( $r = 0.055$ ,  $P = .085$ ) or LL meibograde ( $r = 0.018$ ,  $P = .582$ ) in the MGD group. Similarly, in the control group, age was not correlated with UL meibograde ( $r = -0.294$ ,  $P = .066$ ) or LL meibograde ( $r = -0.025$ ,  $P = .879$ ). The average meibograde for all 4 eyelids in a patient correlated with age ( $r = 0.286$ ,  $P < .001$ ) in MGD patients, but not in the control group ( $r = 0.194$ ,  $P = .413$ ).

## DISCUSSION

IN THE PRESENT STUDY, WE INVESTIGATED MG morphology assessed by meibography in relation to common clinical tests used to evaluate DED. We found significantly higher MG loss in MGD patients compared

to controls. Our subjective meibograde grading system showed excellent ability to discriminate between MGD patients and controls. Furthermore, we observed increased tear fluid production in early-phase MGD and thickening of MGs with increasing subjective meibograde scores in MGD patients. Moreover, clinical parameters were weakly correlated to MG morphology and symptoms score was not related to changes in MG morphology.

The varied presentation of MGD makes early detection of MGD development complicated. Therefore, an effective and reliable clinical screening test is strongly needed. Previous studies have evaluated grading of meibography as a reliable clinical test.<sup>31,32</sup> Furthermore, Arita and associates demonstrated the diagnostic ability of their meibography grading scheme to discriminate between patients and healthy subjects.<sup>14</sup> A number of different grading scales have been used in previous studies, including 4-point,<sup>20,33</sup> 5-point,<sup>31</sup> and 7-point grading scales.<sup>34</sup> All of these define the lowest grade (grade or score 0) as no MG loss or no gland dropout. A previous report suggested a cut-off value of 16.9% (upper eyelid) and 28.7% (lower eyelid) in MG loss for the purpose of discrimination

between dry eye and normal eyes.<sup>19</sup> This demonstrates high variability of mild MG loss within the healthy population. Using a grade defined as 0% when such high variability has been demonstrated could potentially create a bias and a weakness in the grading of MG loss. Therefore, we employed a modified 4-point grading scale for MG loss.<sup>20</sup> More specifically, we use even increments of quartiles, including grade 0 being defined up to 25% MG loss, in an attempt to account for normal variation within the healthy population. Our modified meibograde showed excellent ability to differentiate between MGD and healthy subjects, with high sensitivity and specificity, using an average meibograde value for all 4 eyelids. These results are useful to establish the clinical application of meibography imaging in early detection of MGD by evaluation of MG loss.

Schirmer I test indicate changes in tear fluid production that are diagnostic of aqueous-deficient dry eye. In the current study, in both patient and control groups, the mean value of Schirmer I test was well within normal range and above the lower cut-off values for DED (<5.5 mm). Neither the patients nor the controls appear to have aqueous-deficient dry eye.<sup>1</sup> Furthermore, there were no significant differences between Schirmer I values in the patient and control group. Arita and associates reported higher Schirmer I values in MGD patients in comparison to healthy subjects, and proposed increased tear fluid production as a compensatory response in MGD patients.<sup>35</sup> However, we did not observe higher Schirmer I values in MGD patients compared to normal controls. Nevertheless, our study showed that Schirmer I was increased in patients with UL meibograde 1, compared to patients with meibogrades 0, 2, or 3. Thus, we suggest that a potential compensatory increase in tear fluid production that occurs owing to MG loss and tear film lipid layer dysfunction, may only appear in early stage MGD. Contrary to the findings by Arita and associates,<sup>35</sup> we noted a trend that may indicate a reduction in tear fluid production in severe MGD. This is in line with studies reporting that MGD and aqueous-deficient DED may overlap and occur simultaneously in DED.<sup>36</sup> A possible explanation is that either the MGs or the lacrimal glands are exhausted after compensatory attempts in advance stages of MGD, secondary to either MGD or aqueous-deficient DED. However, as this was only noted for the upper eyelid, and Schirmer I measures reflex tear fluid production, confounding factors, such as inflammation, may contribute to this observation.

We found that MG thickness increased with progressive MG loss. Several studies previously hypothesized that MG thickness is observed as a compensatory or secondary response.<sup>18,23,37</sup> Studies also reported dilation of the secretory acini in MGs, indicating a cellular response to MG loss and reduced meibum secretion or increased meibum demand.<sup>37,38</sup> The mechanisms underlying the dilation of the secretory acini in MGs are unknown. Compensatory cellular repair mechanisms could result in hyperplastic acini with increased lipid production.<sup>18,39</sup>

Whereas the increase in thickness and dilation of secretory acini may increase meibum production and secretion, this process could be exhausted over time. Accordingly, we found that despite the increasing MG thickness, MG expressibility gradually worsened with higher meibograde. Interestingly, we also observed that the interglandular space increased in patients with higher meibogrades, especially those with meibograde 3. Thus, despite the increasing thickness of single MGs, the space between MGs also increased. An explanation could be that MGD patients could have atrophy of entire glands.<sup>18</sup> This would allow for the remaining MGs to thicken and the interglandular space to increase, indicating progressive total atrophy of the MG tissue. Together, these findings demonstrate that MG thickening is an inadequate response to progressive glandular atrophy.

A previous report revealed that MG loss is directly linked to reduction of the lipid layer thickness in the tear film,<sup>40</sup> which leads to shorter TFBUT. Thinner lipid layer thickness is associated with increased friction during blinking.<sup>41</sup> We observed reduced TFBUT in our patient group, and also significantly shorter blink intervals in comparison to the healthy subjects. Blinking rate was suggested as a compensatory measure in DED, as blinking aids in distribution of tear fluid across the ocular surface.<sup>42,43</sup> Furthermore, hyperkeratinization of MG orifices is thought of as a major contributor of MGD pathogenesis. The obstruction of the gland orifice would lead to an increase in intraductal pressure and consequently dilation of MGs. More frequent blinking and increased friction can worsen the increase in intraductal pressure caused by gland obstruction.<sup>2,18</sup> Thus, we speculate that the increase in MG thickness could be a consequence of the increasing intraductal pressure rather than the lack of meibum secretion,<sup>2,18,44</sup> making it a secondary, nonfunctional change. On the other hand, in the present study we demonstrated that MG thickness was inversely correlated to tear film osmolarity and meibum expression, which may indicate a functional compensatory aspect to the increase in MG thickness. Taken together, our findings may support a combined explanation for increased MG thickness, which can be attributed to a compensatory response owing to increased meibum demand and/or dilation caused by increased intraductal pressure.

The tear film osmolarity tends to be elevated in MGD patients.<sup>45–48</sup> However weak, the relationship between gland thickness and tear film osmolarity may point to a functional increase in MG thickness that could help to maintain osmolarity levels. Arita and associates also proposed that increased tear fluid production, which we found in our study in early-phase MGD, might contribute to preventing elevated osmolarity levels.<sup>35</sup> Additionally, the increase in MG thickness seems to have a weak positive effect on meibum expression. Thus, the increase in MG thickness, together with the increase in tear fluid production, may play partial roles in attempting to maintain ocular surface homeostasis with the onset of MGD.

Most clinical dry eye tests demonstrated subtle changes with increasing meibograde. OSDI scores were also independent of MG loss in the patient group. Previous studies have shown that the meibum reservoir in humans is much greater than the amount of meibum actually used in the tear film lipid layer.<sup>24,25</sup> This may partly explain why the tear meniscus height in MGD patients is comparable to that of healthy subjects.<sup>41</sup> The lack of major changes in clinical dry eye test results and symptoms with increasing meibograde could therefore be attributable to the unused meibum reservoir in the patients included in our study. However, the patients in this study have test results beyond cut-offs for MGD. Thus, it is likely that this reservoir has already been depleted. Further development of MGD could at this point be slow in nature. On this basis, we propose that there is a threshold for MGD, with regard to MG loss, after which symptoms occur, which also explains the existence of an asymptomatic MGD population.<sup>4,7,49,50</sup>

In this study, we observed that MG morphology was only weakly correlated to clinical dry eye tests. Contrary to our findings, Pult and associates reported significantly stronger correlation coefficients.<sup>12,19</sup> Most studies have, however, reported inconsistent results with regard to the relationship between MG loss and clinical tests.<sup>11,13–17,21,22</sup> Consistent with our findings, these studies also show that the strongest relationships were seen between MG morphology and meibum expression. Interestingly, we found no correlations between meibum quality and MG morphology. It may be noted, however, that meibum expression only indicates

whether a gland is able to secrete meibum, and not the amount of meibum secreted. Furthermore, in this study, the quality of meibum is only detected grossly by eye, and the actual lipid composition of the meibum remains unexplored. Changes in MG morphology may not directly affect the ability of a gland to secrete, or the macroscopic quality of the meibum. It could, however, have an impact on the amount of meibum secreted and/or the microscopic composition of the lipid layer. Furthermore, as inexpressible glands are given a score of 0 for meibum quality, this may contribute to a bias in the meibum quality scores among patients with low meibum expressibility scores.

In conclusion, MG loss appears to be the hallmark of MGD development, and the subjective meibograde score was an effective diagnostic tool for MGD. Our meibograde demonstrated excellent ability to differentiate MGD patients and healthy subjects, and could be used in clinical investigation and screening of early-stage MGD. Changes in MG morphology were only weakly associated with clinical signs and dry eye test values, and were not linked to symptom score. Development of MGD could be attenuated by compensatory mechanisms, such as increased aqueous tear fluid production and increased thickness of MGs, making early detection of MGD difficult by standard clinical measures of dry eye, but possible using morphologic MG analysis. Investigations of MG morphology visualized by meibography therefore represent a complementary clinical parameter with diagnostic potential.

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