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Meibomian Gland Contrast Sensitivity and Specificity in the Diagnosis of Lipid-deficient Dry Eye: A Pilot Study

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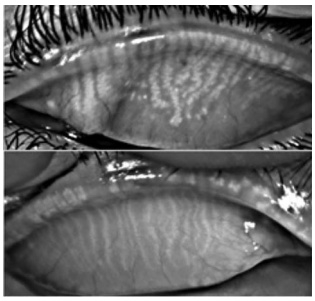
SIGNIFICANCE: Lipid deficiency due to meibomian gland (MG) dysfunction is believed to account for the vast majority of patients with dry eye compared with aqueous deficiency. Clinicians commonly evaluate MG length to determine a disease, but our research with isotretinoin users suggests that MG contrast is also an important characteristic to consider.

PURPOSE: This study aimed to determine the sensitivity and specificity of MG contrast for the diagnosis of lipid-deficient dry eye (LDDE).

METHODS: This case-control study used demographic data, Standard Patient Evaluation of Eye Dryness (SPEED) scores, average tear lipid layer thickness (TLLT), fluorescein tear breakup time (FTBUT), upper eyelid meibography images, and meibum quality and quantity scores for individuals with LDDE (SPEED score ≥10 and TLLT ≤35 interferometric color units) and normal individuals (SPEED ≤2 and TLLT ≥80 interferometric color units).

RESULTS: Thirty-one eyes of 22 controls (mean ± SD age, 22.7 ± 5.5 years) and 13 eyes of 12 cases (mean ± SD age, 43.9 ± 17.2 years) were included. Normalized MG contrast was significantly correlated with FTBUT ($r = 0.35$, $P = .02$), percent MG atrophy ($r = –0.50$, $P < .001$), and SPEED scores ($r = –0.49$, $P < .001$). The receiver operating characteristic curve for LDDE diagnosis classifiers MG contrast, MG atrophy, and meibum quantity score had areas under the curve of 0.83, 0.64, and 0.73, respectively. Meibomian gland contrast cutoff at 28.3 intensity units yielded optimal correct classification of subjects (84.1%; sensitivity, 0.69; specificity, 0.90). Cases had shorter FTBUT ($P < .001$), worse meibum quality ($P = .02$) and quantity ($P = .02$) scores, and lower MG contrast ($P < .001$) compared with controls. Subjects with low MG contrast (≤28.3) had 14.9 higher odds of having LDDE (95% confidence interval, 2.84 to 78.4) compared with subjects with high MG contrast (>28.3).

CONCLUSIONS: Meibomian gland contrast correlates well with clinical parameters and symptoms, shows good sensitivity and excellent specificity for diagnosing LDDE, and can be a useful diagnostic parameter for monitoring MG changes due to age, disease, or intervention.



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Meibomian gland dysfunction is believed to be the most prevalent form of dry eye disease.[1] Meibomian gland dysfunction is an abnormality of the meibomian glands characterized by altered quality or quantity of glandular secretions and can present as either hypersecretory or hyposecretory forms.[2] Hyposecretory meibomian gland dysfunction is a state of decreased lipid secretion associated with obliterated meibomian gland ducts, orifice obstruction due to hyperkeratinization, or other pathologies without concurrent remarkable obstruction.[2] A state of compromised meibum quality or quantity can result in lipid-deficient dry eye.

Meibomian gland expression allows for the assessment of glandular secretions upon exiting the gland duct at the eyelid margins, whereas tear film interferometry allows for the visualization of lipid quality and quantity in the tear film. Meibomian gland expressibility score <12 units and lipid layer thickness <30 nm are generally considered abnormal.[3,4] Meibography has long been used to evaluate gland structure, or atrophy, either subjectively using various published grading scales or objectively using software.[5–9] However, the correlation between meibomian gland atrophy and other clinical parameters or symptoms has not always been consistent.[9–11]

Reports of patients on isotretinoin suggest that meibomian glands can change in ways other than shortening.[12] Rather, these patients present with fading of glands during their treatment period.[12] To properly monitor the changes in these patients, we recently evaluated the repeatability of meibomian gland contrast using the OCULUS Keratograph 5M (OCULUS, Inc., Arlington, WA) and found it to be a repeatable, objective measure that can potentially be used to assess meibomian gland function.[12] It is possible that patients using other medications, including antiandrogens and post-menopausal hormone therapy, or with systemic conditions, including androgen deficiency, atopy, psoriasis, and rosacea, can present with similar changes in their meibomian glands.[13]

In this study, we aimed to conduct a pilot study to evaluate the significance of meibomian gland contrast among a group of lipid-deficient dry eye cases and controls. First, we evaluated the differences in ocular surface parameters, including meibomian gland contrast, between cases and controls. We also determined the potential validity of using meibomian gland contrast as a classifier for lipid-deficient dry eye by developing a receiver operating characteristic curve and estimating the sensitivity and specificity

at the optimal cut point for meibomian gland contrast in the diagnosis of lipid-deficient dry eye.

METHODS

Subjects

This was a retrospective, noninterventional study in which clinical data and meibography images were extracted from the Ocular Surface Study database at the Clinical Research Center at the University of California, Berkeley, School of Optometry. Subjects were filtered and classified based on two parameters: Standard Patient Evaluation of Eye Dryness score and average tear lipid thickness (LipiView; Johnson & Johnson, Santa Ana, CA). This was a case-control study in which individuals with lipid-deficient dry eye (cases) were those who had severe symptoms (Standard Patient Evaluation of Eye Dryness score ≥10) and thin average tear lipid thickness (≤35 interferometric color units), and normal individuals (controls) were those with very mild to no symptoms (Standard Patient Evaluation of Eye Dryness score ≤2) and thick average tear lipid thickness (≥80 interferometric color units). The Standard Patient Evaluation of Eye Dryness score cutoffs were adapted from previously reported classification and also represent the upper and lower quartiles of this study population.[3] For tear lipid thickness, the lower cutoff was based on the finding by Svitova and Lin[4] that tear film lipids reach maximum surface pressure at thicknesses greater than approximately 30 nm; rounding up to the lower 10% of the study population, we set our limit to 35 nm. The upper cutoff for tear lipid thickness was set to represent the upper quartile of the study population, which was similar to that previously reported.[3] Both eyes from patients were considered if inclusion criteria were met because tear lipid layer can be clinically different between eyes. The data from the database were collected after obtaining written informed consent from all study participants. The study adhered to the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley, Office for the Protection of Human Subjects.

Ocular Surface Study Database

The Ocular Surface Study database consists of uniformly collected data on demographic variables, medical history, symptoms, and ocular surface data from 345 subjects. For the purposes of this study, the following data were extracted from the database: demographic variables, ocular and systemic medical history, Standard Patient Evaluation of Eye Dryness scores, average tear lipid thickness measured with the LipiView, fluorescein tear breakup time, Schirmer's tear test results, upper eyelid meibography images (OCULUS Keratograph 5M; OCULUS, Inc.), and upper eyelid meibomian gland expression quality and quantity scores. Meibomian gland expression was performed with the Korb Meibomian Gland Evaluator to assess both the quantity and quality of the expressed meibum. Evaluation, scoring, and calculations for meibum quality and quantity scores were previously described.[14,15] Percent meibomian gland atrophy and meibomian gland contrast values of upper eyelid meibography images were also measured using a previously described method.[12,16] In brief, meibography of the upper and lower eyelids from both eyes of the study participants were captured with the OCULUS Keratograph 5M (OCULUS, Inc.), which produces two images: raw and processed. The OCULUS-processed images have increased contrast between the meibomian glands and the surrounding tissues and are the ones analyzed in this study. Using Fiji (version 2.0.0-rc-59/1.51k; U.S. National Institutes of Health, Bethesda, MD),[17] an image processing package (ImageJ with plugins), mean pixel intensity (gray scale, 0 to 255) was measured of segmented lines drawn along the central five meibomian glands and along the background regions between the meibomian glands measured. The difference between mean intensity along the meibomian glands and the mean intensity along background regions between the meibomian glands was defined as contrast.

Statistical Methods

Robust (clustered) logistic regression models using the Huber-White standard error estimator clustered by subject (Stata/IC 14.0; vce(cluster) option; StataCorp LLC, College Station, TX) to account for within-subject correlations between eyes were used to evaluate differences in clinical parameters between the case and control groups. With meibomian gland contrast as a classifier, a receiver operating characteristic curve was developed, and the area under the curve was calculated while controlling for correlations between eyes using participant IDs as sampling clusters. Sensitivity, specificity, and classifier cut point at the optimal correct classification of cases and controls were estimated with one-eye (randomized) analysis. The area under the curve was also estimated with percent meibomian gland atrophy and meibum quantity score as classifiers for comparison. Because higher values of meibomian gland contrast and meibum quality scores represent better health status, the reciprocal (inverse) of these values was used to calculate the area under the curve and plot receiver operating characteristic curves, which require that higher values of classifiers represent higher risk. The optimal meibomian gland contrast cutoff was determined using the ROCTAB command, which lists various cutoff values and the corresponding percentage of patients correctly classified and the resulting sensitivity and specificity values. The cutoff with the maximum percentage of correctly classified patients will be reported. All analyses were conducted with Stata (Stata/IC 14.0; StataCorp LLC).

RESULTS

Subjects

Of the 345 subjects in the Ocular Surface Study database from 2012 to 2018, 31 eyes of 22 subjects (including both eyes for 9 subjects) fulfilled the criteria to be controls, and 13 eyes of 12 subjects (including both eyes for 1 subject) fulfilled the criteria to be cases. Their demographic data are presented in Table 1. In general, cases were more likely to be older, male, and nonusers of contact lenses but were similar to controls with respect to race.

Case versus Control

Cases showed significantly worse ocular surface signs for all characteristics observed in this study, except for Schirmer's tear test (Table 2). Cases had, on average, 11.9-unit lower meibomian gland contrast ($P < .001$), 18.9 higher percent meibomian gland atrophy ($P = .04$), 7.6-unit lower meibum quality ($P = .04$) scores, 7.7-unit lower meibum quantity ($P = .02$) scores, and 9.5-second shorter fluorescein tear breakup time ($P < .001$) compared with controls. The difference in Schirmer's tear test results was not statistically significant ($P < .77$). Because of the difference in mean age between the case and control groups, post hoc analysis was done to control for age, and as a result, percent meibomian gland atrophy and meibum quantity were no longer different between

TABLE 1. Characteristics of cases and controls

	Control (n = 22; 31 eyelids)	Case (n = 12; 13 eyelids)
Age, mean ± standard deviation (y)	22.7 ± 5.5	43.9 ± 17.2
Sex, count (%)		
Male	5 (23)	7 (58)
Female	17 (77)	5 (42)
Race, count (%)		
White	5 (23)	4 (33)
Asian	10 (45)	5 (42)
Other	7 (32)	3 (25)
Contact lens, count (%)		
Users	11 (50)	4 (33)
Nonusers	11 (50)	8 (67)

groups (Table 2). The median percent meibomian gland atrophy values were 24.3% (range, 0 to 91.5%) among cases and 18.3% (range, 0 to 42.9%) among controls, with the lower quartiles for each group having ≤13.0 and <11.0%, respectively, and the upper quartiles having ≥56.2 and >28.0%, respectively.

Sensitivity and Specificity of Meibomian Gland Contrast

The receiver operating characteristic curve with the inverse of meibomian gland contrast as a classifier for lipid-deficient dry eye diagnosis resulted in an area under the curve of 0.83 (95% confidence interval, 0.75 to 0.99; Fig. 1, Table 3). Fig. 1 and Table 3 show the receiver operating characteristic curve and area under the curve for meibomian gland contrast, compared with percent meibomian gland atrophy and (inverse of) meibum quantity score (areas under the curve of 0.66 and 0.73, respectively) in diagnosing lipid-deficient dry eye. For a range of meibomian gland contrast values, the percentage of correctly classified patients, as well as both sensitivity and specificity, was evaluated. A meibomian gland contrast cut point of 28.3 intensity units yielded optimal correct classification of study subjects at a rate of 85.3%, with a sensitivity of 0.67 and a specificity of 0.95. Using a cutoff of 28.3 intensity units, 8 (73%) of 11 case eyes had low-contrast meibography images, and 28 (85%) of 33 control eyes had high-contrast meibography images.

DISCUSSION

Meibography has been used as a tool to support the diagnosis of meibomian gland dysfunction. These images can offer clues about the etiology and severity of disease and are often evaluated for gland length, dilation, or presence of blockage. Evidence from patients on isotretinoin suggests that decreased lipid production results in reduced gland intensity on meibography, not gland shortening.[12] This pilot study aimed to explore the significance of meibomian gland contrast for the diagnosis of lipid-deficient dry eye, which was defined in this study as the combination of thin tear lipid layer (≤35 interferometric color units) and severe symptoms (Standard Patient Evaluation of Eye Dryness score ≥10). As previously reported, the study found that lipid-deficient patients had significantly worse ocular surface characteristics than did controls (thick tear lipid layers [≥80 interferometric color units], mild to no symptoms [Standard Patient Evaluation of Eye Dryness score ≤2]), in all areas evaluated in this study, except for Schirmer's test.[18] The study also determined that meibomian gland contrast may be a good diagnostic indicator for lipid-deficient dry eye with good sensitivity and excellent specificity.

The study population was selected based on a combination of findings from previous clinical studies and the characteristics of the source database. Blackie et al.[3] reported that, in their study population, the lower quartile of thickness among patients with severe dry eye was ≤60 nm, and the upper quartile was ≥75 nm. Svitova and Lin[4] reported that maximum surface pressure was achieved once tear lipid thickness reached 25 to 30 nm. In this study population, the lower quartile of tear lipid thickness among patients with severe dry eye was <46 nm, but if we also consider the findings from Svitova and Lin, which suggest that more compromised tear films have thicknesses <25 to 30 nm, we determined that ≤35 nm (fewer than 10% of patients with severe dry eye) was a good compromise. The upper quartile of tear lipid layer thickness among patients with mild to no symptoms in this study population was 80% and explains why we chose this as the cutoff for controls. Larger study populations may be warranted to determine the optimal cutoff points.

In the side-by-side comparison of patients with lipid-deficient dry eye and controls (Table 2), all ocular surface parameters investigated in this study were statistically significantly worse in cases

TABLE 2. Comparison of ocular surface characteristics between cases and controls

	Case (n = 12; 13 eyelids), mean ± SD	Control (n = 22; 31 eyelids), mean ± SD	Mean difference (95% CI)	*P**	*P*†
Meibomian gland contrast	25.2 ± 8.8	37.1 ± 8.7	−11.9 (−17.7 to −6.1)	<.001	.004
Percent meibomian gland atrophy	36.9 ± 31.8 (range, 0 to 91.5)	18.0 ± 11.9 (range, 0 to 42.9)	18.9 (0.98 to 36.9)	.04	.19
Meibum					
Quality score	17.2 ± 10.5	24.7 ± 11.0	−7.6 (−14.7 to −0.4)	.04	.001
Quantity score	11.3 ± 9.4	19.0 ± 10.3	−7.7 (−14.2 to −1.1)	.02	.11
Fluorescein tear breakup time (s)	4.2 ± 2.8	13.7 ± 10.8	−9.5 (−14.3 to −4.7)	<.001	.001
Schirmer's I (mm)	26.4 ± 10.9	25.2 ± 12.0	−1.2 (−9.2 to 6.8)	.77	.44

**P*-value using the univariable linear regression model with Huber-White standard error estimator clustered by subject. †*P*-value using the multivariable linear regression model controlling for age with Huber-White standard error estimator clustered by subject. CI = confidence interval; SD = standard deviation.

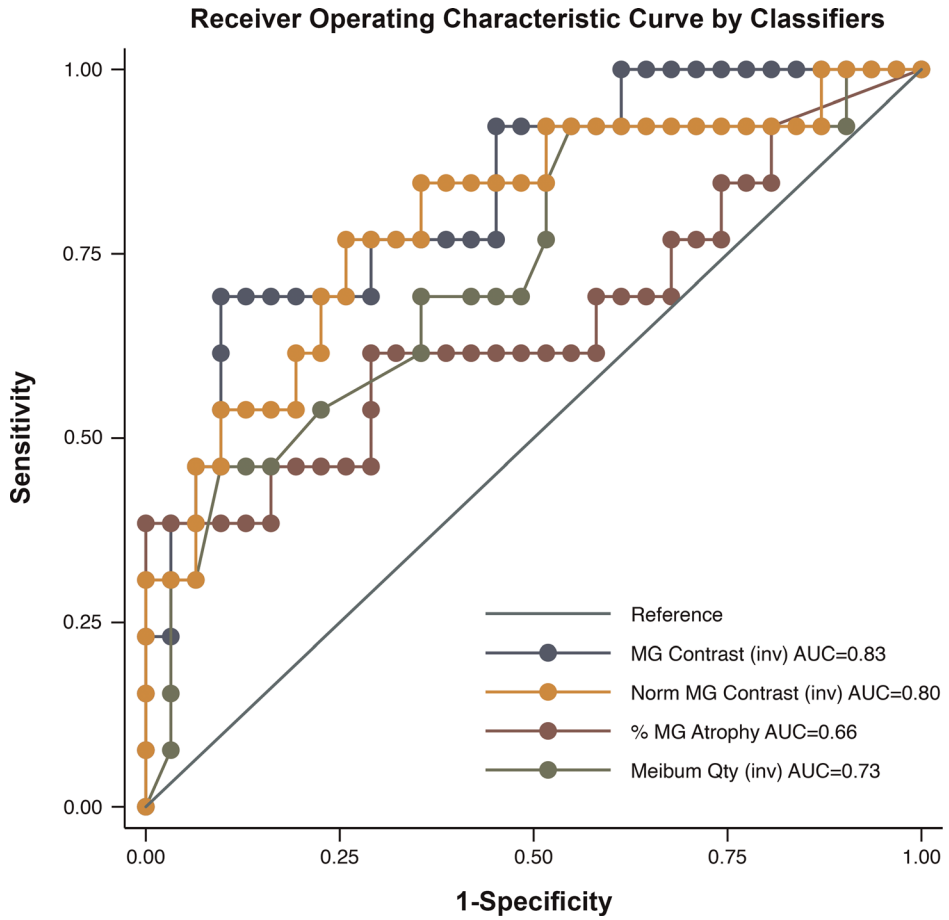

FIGURE 1. Receiver operating characteristic curves for meibomian gland contrast, normalized meibomian gland contrast, percent meibomian gland atrophy area, and meibum quantity scores in the diagnosis of lipid-deficient dry eye.

compared with controls. Cases had poor meibum quality and lower meibum quantity upon expression with a Korb Meibomian Gland Evaluator, which applies a standard pressure. The meibum quality score is a gross evaluation of the meibum appearance with classifications of clear, cloudy, or inspissated, whereas the quantity classifications were copious, moderate, or minimal.[14,15] Such inexact measures are subject to misclassification, but in this study population, there was a distinct difference between the study groups. Cases also had significantly lower fluorescein tear breakup time, supporting the hypothesis that compromised meibomian gland secretions can lead to downstream effects on tear film (shorter tear breakup time) and symptoms.[19,20] Lastly, meibomian gland contrast was approximately 12 units lower in cases than in controls, which is just outside the range of normal intrasubject variability for OCULUS Keratograph 5M, suggesting that this is also clinically significant.[12] Interestingly, cases averaged 19% higher meibomian gland atrophy compared with controls, which is not clinically significant because many meibomian gland scoring scales require an estimated difference of 25 to 33% between a grade 0 and a grade 1.

TABLE 3. Summary of ROC AUC for potential classifiers of lipid-deficient dry eye (n = 44)

	ROC AUC	95% Confidence interval
Meibomian gland contrast*	0.83	0.70–0.96
Normalized meibomian gland contrast*	0.80	0.64–0.95
Percent meibomian gland atrophy	0.66	0.45–0.86
Meibum quantity score*	0.73	0.55–0.90

*Inverse values were used to generate ROC curves. AUC = area under the curve; ROC = receiver operating characteristic.

The relationship between various meibomian gland characteristics and symptoms and ocular surface signs has been assessed, but there have been no reports on the relevance of meibomian gland contrast. Part of the challenge is that there are many tools to visualize meibomian glands, and depending on the tool used, there may be a lot of variability. In this study, all meibography images were captured with the OCULUS Keratograph 5M, which limits the generalizability of these results. According to previously published results on the repeatability of meibography images captured with the OCULUS Keratograph 5M, variability in meibomian contrast for a single subject at different visits, head positions, and lighting conditions was not greater than 12 units of contrast.[12] The differences in meibomian gland seen between cases and controls in this study exceed the range of normal variability and can be interpreted with some confidence that the differences seen are clinically significant.

The meibography results suggest that evaluating both meibomian gland atrophy and contrast is important in trying to diagnose and understand the etiology of symptoms. After controlling for age, the results confirmed meibomian gland contrast and meibum quality to be significantly different between the case and control groups (Table 2). Schirmer's I test remained nondiscriminatory between groups, in agreement with published works that have shown the limitations of Schirmer's I test.[21–24] The variances of meibomian gland and fluorescein tear breakup time are vastly different between study groups, whereas other parameters remain similar between groups. The large variability of meibomian gland atrophy is, in part, responsible for why it is not a good discriminator. The wide range of variability of tear film stability reflects the widely known natural lability of the healthy tear film. For example, when considering the images alone in Fig. 2, one might mistakenly guess that the image for the control subject was a case due to the moderate atrophy. However, as indicated below the images, the individuals with same or less atrophy but lower contrast were more symptomatic and presented with worse clinical signs. Lipids contained in meibum are believed to be highly reactive to infrared light, giving it the high intensity or contrast.[25] Fig. 2 suggests that even moderate meibomian gland atrophy may not affect downstream ocular surface parameters or induce symptoms. If meibomian gland contrast is high enough, we hypothesize that whatever remains in the meibomian gland duct can be sufficient to maintain good ocular surface health and stave off any symptoms of discomfort or dryness. However, systemic changes resulting in altered meibum quantity or composition can adversely impact ocular surface health and symptoms, regardless of gland length.

The receiver operating characteristic curve with the inverse of meibomian gland contrast as a classifier for lipid-deficient dry eye diagnosis resulted in an area under the curve of 0.83, suggesting that meibomian gland contrast may be a good diagnostic test for the disease (Fig. 1, Table 3). In this small pilot sample size, meibomian gland contrast yielded a better area under the curve than percent meibomian gland atrophy (area under the curve, 0.66; poor) and the inverse of expressed meibum quantity (area under the curve, 0.73; fair). Meibum production drastically decreases in individuals treated with isotretinoin, as intended by the treatment, and is apparent with gland expression.[12] However, the characteristic meibography for those undergoing isotretinoin treatment shows no gland shortening/atrophy despite the decreased availability of meibum upon gland expression. It is possible that lengthier treatment durations or extremely high dosages could result in gland atrophy, but the typical course of isotretinoin treatment seems to worsen meibomian gland expression and contrast but not length.[12,26] We suspect that similar meibography findings may also be characteristic of other systemic conditions associated with meibomian gland dysfunction or sebaceous gland atrophy, such as atopic dermatitis, psoriasis, and androgen deficiency.[27]

Given that all clinical factors, except for Schirmer's test, were statistically significantly different between study groups, the value of meibomian gland contrast may be unclear. Meibomian gland contrast can lend greater support to the diagnosis, but more importantly, these results suggest that meibomian gland atrophy should not be the only characteristic analyzed from meibography images. Changes in meibomian gland contrast do not always correlate with changes in gland length, as previously reported in our isotretinoin case.[12] Of note, after controlling for age in the post hoc analysis, there was no difference in gland atrophy or meibum quality between case and control, but meibomian gland contrast continued to be significantly different between the groups. Therefore, we think that there is value in assessing this characteristic when analyzing meibography images.

This study had several limitations. The sample size represents the exploratory nature of this study. As evidenced by the stepwise appearance of the receiver operating characteristic curve, a larger sample size is warranted to confirm the findings of the present study. Also, the generalizability of these results is limited because the classification criteria were specifically based on the Standard Patient Evaluation of Eye Dryness score, LipiView measurements, and OCULUS Keratograph 5M imaging. Further research is warranted to determine if these relationships would hold true with meibography from other instruments or if the criteria for case and control are expanded.

In summary, meibomian gland contrast might be a good indicator of lipid-deficient dry eye, which was defined in this study as presence of thin tear lipid layers (≤35 interferometric color units) and severe symptoms (Standard Patient Evaluation of Eye Dryness score ≥10). Meibomian gland contrast offered good area under the

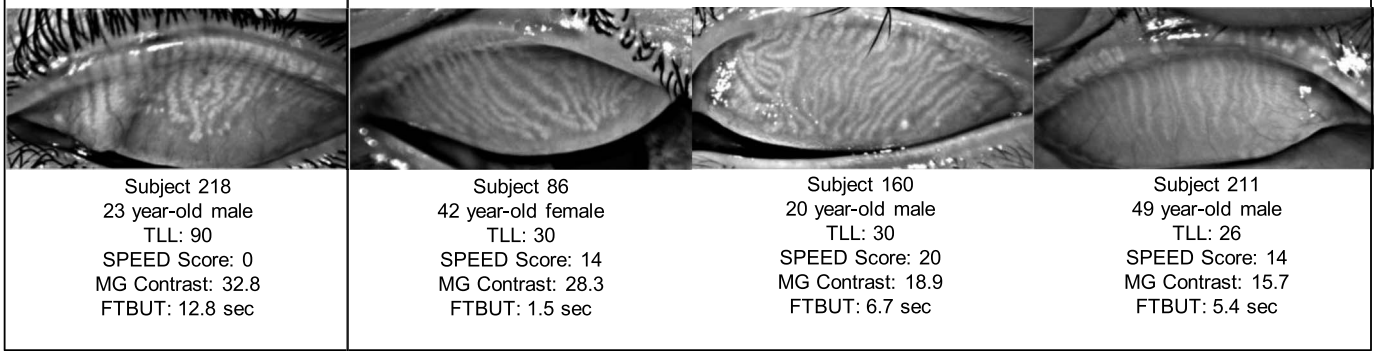

FIGURE 2. Meibography images for a control subject (leftmost) and cases. Despite the presence of meibomian gland (MG) atrophy in the control subject, tear lipid layer (TLL) was thick and provided good tear film stability (fluorescein tear breakup time [FTBUT]) and no dryness symptoms (Standard Patient Evaluation of Eye Dryness [SPEED] score). Cases on the right have minimal atrophy but have thin TLL and severe symptoms. All cases had MG contrast less than or equal to 28.3 intensity units, the cutoff for optimal sensitivity and specificity for the diagnosis of lipid-deficient dry eye in this study.

curve, good sensitivity, and excellent specificity for the diagnosis of lipid-deficient dry eye in this pilot study. Additional studies are needed, with larger sample sizes, to validate these meibomian gland contrast metrics. Although it is recommended that meibum expression and percent meibomian gland atrophy continue to be evaluated in patients with dry eye, meibomian gland contrast should also be considered, particularly for symptomatic patients with minimal percent meibomian gland atrophy.

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