Original Article

Meibography signal intensity as a novel image biomarker for meibomian gland function: evidence from cross-sectional and longitudinal analyses

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Background: Meibomian gland dysfunction (MGD), one of the most common ocular surface diseases, can induce dry eye and reduce patients' quality of life. Methodological limitations have resulted in contradictory interpretations of gland function. This study sought to investigate the correlation between meibography signal intensity (SI) and meibomian gland (MG) function and to validate an MGD classification strategy based on different levels of SI.

Methods: A multicenter, cross-sectional analysis was conducted on 817 eyes from 361 patients with MGD and 52 healthy controls. Additionally, 78 eyes from 39 patients with MGD who had undergone LipiFlow treatment were recruited for longitudinal analyses. The SI value was obtained via meibography using an automated analyzer, and all participants underwent ocular surface examinations. A cross-sectional analysis was performed to determine SI distribution and its relationship to clinical characteristics via a generalized estimating equation model. Longitudinal analyses were conducted on the treatment cohort using a mixed-effects model to explore the outcome in different SI levels.

Results: Regression analysis revealed significant correlations between SI and lipid layer thickness (β=0.016), meibum expressibility (β=−0.676), meibum quality (β=−0.251), and fluorescein-stained tear-film break-up time (FBUT) (β=0.064) (all P values <0.001 for the above associations). Low-level SI MGD cases exhibited the most severe clinical signs, including the worst meibum expressibility (16% for level 3) and quality scores (19% for level 3), the shortest FBUT (3.82±0.13 s), and the thinnest lipid layer (65.68±2.58 nm), (all P values <0.05, respectively). Patients with medium SI showed the lowest ocular surface disease index (OSDI) value (26.64±1.06), the longest FBUT (4.56±0.08 s), and the thickest lipid layer (80.20±2.90 nm). After treatment, the high SI values reduced significantly at each follow-up point compared to baseline (all P values <0.05). The medium SI group demonstrated the greatest improvement in symptoms and signs, followed by the high SI group, and the low SI group.

Conclusions: Automated measurements of SI can effectively reflect MG secretory activity. The proposed low, medium, and high SI classifications represent different functional subtypes of MGD.

Keywords: Meibomian gland dysfunction (MGD); signal intensity (SI); meibography; image biomarker

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Introduction

Meibomian gland dysfunction (MGD), one of the most prevalent ocular surface diseases that significantly degrades patients' quality of life, is characterized by terminal duct obstruction and/or abnormal glandular secretion leading to tear film instability and subsequent dry eye (1). Several diagnostic criteria of MGD have been developed based on meibum quality and expressibility using physical force applied to the eyelid (2). A classification system based on meibomian gland (MG) function was recommended by the latest International Workshop on MGD to best address the needs of clinicians and researchers (1). This system includes low-delivery MGD, which can be further divided into hyposecretory and obstructive subtypes, as well as high-delivery MGD (hypersecretory MGD) (1). However, meibum quality and expressibility are considered unsatisfactory alternative markers for MG secretion due to their subjectivity and inexactness in scoring (3,4). Additionally, evaluating only a limited number of centrally located glands may hinder the accurate evaluation of MG function (5), and it is impossible to evaluate meibum quality if a gland is completely obstructed. Lipid layer thickness (LLT) is another commonly used indicator that can also reflect the capacity of MG secretion, but it can be influenced by the instantaneous eye-blink pattern (6), corneal erosions (7), and the cycle of glandular secretion (8), which also leads to difficulties in interpretation. Overall, there is inconsistency among the currently employed gland function evaluation methods and a lack of consensus regarding the differentiation of MGD subtypes (1,9).

Meibography can visualize the morphology of MGs via the use of an infrared transmitting filter to capture the shapes of the MGs *in vivo*. Although morphological changes are useful in grading gland dropout, they may not provide accurate and direct estimates of MG function (10). According to the principle of infrared imaging, lipids from meibum are highly reactive to infrared light, presenting a higher signal intensity (SI) in meibography compared with conjunctiva (11,12). Infrared filters are widely used to measure lipid content in biological tissues (11). Several previous clinical observations from meibography also revealed that changes in the activities of secretion and quality of meibum inside MGs could alter the intensity of the glands (10,13). This implies that gland intensity may be a potential indicator of MG secretory function and capable of differentiating between the functional subtypes of MGD. Yeh *et al.* proposed a MG intensity measurement method (14) that is primarily reliant on the manual delineation of glands but is only suitable for high-quality, small-sample image sets and requires a significant amount of manual correction. Our previous study proposed an MG bioimage analyzer (15), which demonstrated good glandular segmentation accuracy in using morphological parameters to assist in MGD grading (16). However, the calculation of meibography SI with software still exhibits significant variability due to the influence of image quality factors such as reflection and shadows (16). Therefore, algorithm optimization and clinical validation on large samples sizes are necessary to determine the relationship between SI and MG function.

This study aimed to develop an automatic imaging analysis method to obtain the meibography SI relevant to the meibum contrast within the MGs. By collecting data from cross-sectional and treatment cohorts, we aimed to clarify the relationship between SI and glandular function and to validate the proposed classification standard for MGD subtypes based on different SI levels. We present this article in accordance with the STROBE reporting checklist (available at https://qims.amegroups.com/article/view/10.21037/qims-24-379/rc).

Methods

Study population

A cross-sectional study was conducted in which 361 patients with MGD and 52 healthy controls without history or condition of ocular or systemic illness were recruited from March 2019 to October 2022 from three hospitals in China (*Figure 1*). This study was approved by the institutional review board of Zhongshan Ophthalmic Center at Sun Yat-

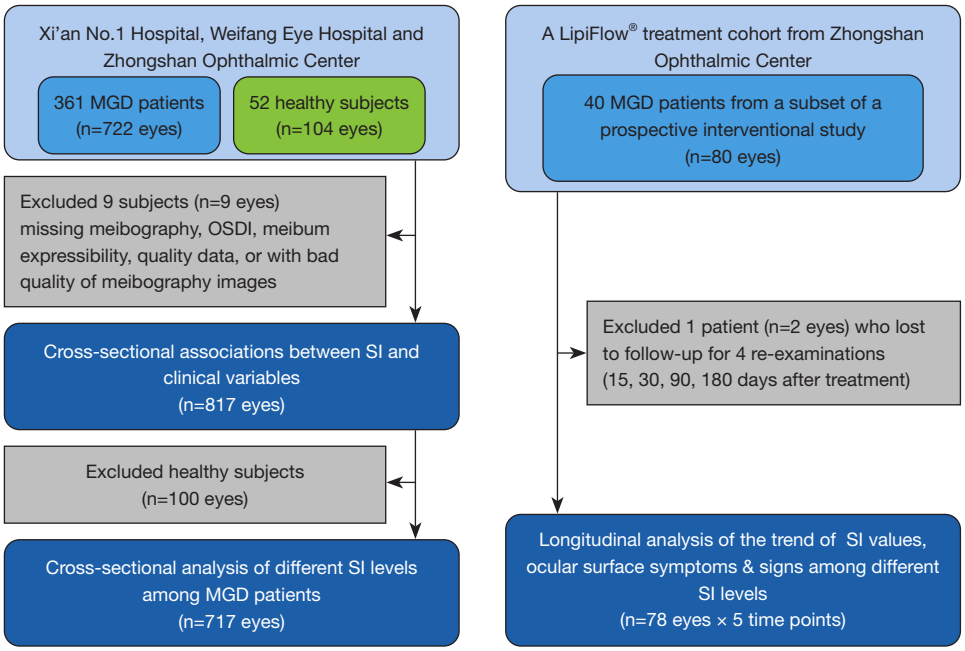

Figure 1 A flowchart of participants included in the cross-sectional and longitudinal analyses from multiple centers. MGD, meibomian gland dysfunction; OSDI, ocular surface disease index; SI, signal intensity.

sen University (IRB-ZOC-SYSU; ID: 2021KYPJ215). All participating institutions were informed and agreed with the study. The participants of the longitudinal analyses were from a LipiFlow treatment subset of a prospective interventional study recruited from October 2020 to October 2022 that was initiated in Guangzhou, China, in July 2019 (ClinicalTrials.gov; identifier: NCT04052841) and approved by IRB-ZOC-SYSU (ID: 2019KYPJ110-4). Informed consent was obtained from all participants prior to data collection, and the study was performed according to the Declaration of Helsinki (as revised in 2013).

The general inclusion criteria for both the cross-sectional and longitudinal studies included individuals between the ages of 18 and 70 years who were willing and capable of participating in the clinical study. The general exclusion criteria were a history of ocular allergies, contact lens wear, ocular surface surgery, trauma, active ocular surface infection, endophthalmitis, viral keratitis infection, Sjogren syndrome, lacrimal gland abnormality, and pregnancy or nursing. Meibography images that were blurred or that exhibited obvious tarsus folds, incomplete exposure, or large hyperreflective areas in the inverted tarsus region were also excluded. Additional exclusion criteria for the longitudinal study encompassed patients with a ≥ two-thirds MG atrophy; abnormalities in ocular surface function or eyelid function; presence of precancerous lesions, cancer, or pigmentation in the eyelid area; continuous use of medications such as tretinoin, isotretinoin, antidepressants, glucocorticoids, and immunomodulators; or recent usage of these medications within the past month. Patients with plans to undergo ocular surgeries within the next 6 months were also excluded. The diagnosis of MGD was based on the presence of an altered quality of expressed secretions and/or decreased or absent expression as follows: (I) score >1 for either meibum quality or expressibility or (II) score =1 for both meibum expressibility and meibum quality (2). The patients with MGD were further evaluated with regard to the MGD severity level and were scored from 1 to 5 according to the 2011 MGD workshop recommendations (2).

All participants completed a comprehensive ocular surface examination during their initial consultation at the clinic. The examinations sequence was as follows: ocular surface disease index (OSDI) symptom questionnaire, meibography images of the upper MG measured with a Keratograph 5M (OCULUS, Wetzlar, Germany), measurement of tear film LLTs measured by LipiView II (Tear Science, Johnson & Johnson, New Brunswick, NJ, USA), assessment of meibum quality and expressibility through the application of pressure by the same trained operators after infrared photography, and measurement of

fluorescein-stained tear-film break-up time (FBUT) through slit-lamp observation. Meibum expressibility was scored from the central five glands of both the upper and lower eyelids as follows: 0 for all five glands, 1 for three to four glands, 2 for one to two glands, and 3 for zero glands (3). Meibum quality was scored on a scale of 0–3: 0 for clear fluid, 1 for cloudy fluid, 2 for cloudy particulate fluid, and 3 foropaque, toothpaste-like meibum (4). The scores of expressibility and quality of meibum from the lower eyelid were used for the diagnosis and grading of MGD, and the scores from the upper eyelid were used for data analysis to ensure that the data were derived from the same anatomical site. In the longitudinal study, a 12-minute LipiFlow thermal pulsation system (TearScience) was conducted after the baseline examinations. Identical re-examinations were performed by a trained and qualified technician at four follow-up visits (15, 30, 90, 180 days) after LipiFlow treatments. During follow-up, each patient was instructed to refrain from any additional treatments apart from the artificial tears they had used before treatment and were advised to minimize exposure to external risk factors that could aggravate MGD, including exposure to air pollution, eye makeup, smoking, alcohol, a high-sugar and high-fat diet, sleep disorders, and use of video terminals exceeding 8 hours daily. If patients are unable to avoid these exposures and the duration of exposure exceeded half of the entire follow-up period, this was recorded.

Since significant variations in SI levels were apparent between binoculus, both eyes were denoted as the case-level result to retain the intereye differences. Of these, 9 eyes were excluded for missing covariates or with bad quality of meibography images, resulting in 817 eyes for a general cross-sectional analysis. For the treatment cohort, 1 patient was excluded for hospitalization for non–study-related illness, resulting in 39 patients with MGD (n=78) for longitudinal analyses. The population samples used for analyses are shown in *Figure 1*.

SI and morphological analysis of meibography

The quantitative SIs were processed and analyzed from meibography images by an updated version of a previous customized automated MG bioimage analyzer (15,16). All images were anonymized and deidentified before the analysis. In brief, based on image contrast enhancement and precise feature-detection algorithms, automated segmentation of the everted tarsal conjunctiva area as the region of interest (ROI) was performed in meibography imaging, which was followed by the identification and segmentation of all glands within the ROI (*Figure 2A*,*2c1-2c4*). The meibography SI was then automatically calculated from the base 10 logarithms of the averaged raw image grayscale value of the segmented intact glands divided by that of the nongland area of the ROI. To reduce the bias of the SI calculation caused by extreme grayscale values from unavoidable hyperreflective spots and eyelashes shadows, a threshold filter with empirically defined upper and lower grayscale limits was added to the grayscale value calculation of both the segmented glands and the remaining nongland conjunctivas within the ROI in order to remove those pixels with abnormal grayscale values. The gland area ratio was calculated from the area of segmented intact glands divided by that of the nongland area of the ROI.

Statistical analysis

Data were analyzed using SPSS version 24.0 software (IBM Corp., Armonk, NY, USA). The normality of the data were assessed using the Kolmogorov-Smirnov test. Normally distributed continuous variables are presented as the mean ± standard error (SE), while categorical variables are reported as counts and frequencies (%). A generalized estimating equation (GEE) was employed for regression analysis to determine the relationships between SI and MG function in the cross-sectional evaluation. The side of the eye was included as an exchangeable within-subject variable to mitigate the impact of intereye correlation on the results, and age and sex were employed as covariates in the GEE model, as this has been suggested to potentially impact the MGs (17). The maximum likelihood method was used for estimation.

The continuous SI values were then categorized into three categories: SI ≤ (the integer closest to the one-third percentile), SI ≥9 (the integer closest to the two-thirds percentile), and 7< SI <9, representing low, high, and medium levels of SI, respectively. Patients with MGD were divided into three groups according to the categorical SI. The GEE analysis was conducted to examine the cross-sectional differences between the three groups. Differences between groups were identified via post hoc pairwise comparison testing within the GEE analysis module, with least significant difference adjustment.

For longitudinal analyses, participants were also divided into three groups according to their baseline SI levels to compare their treatment responses at different follow-up points. A mixed-effects model was employed to assess

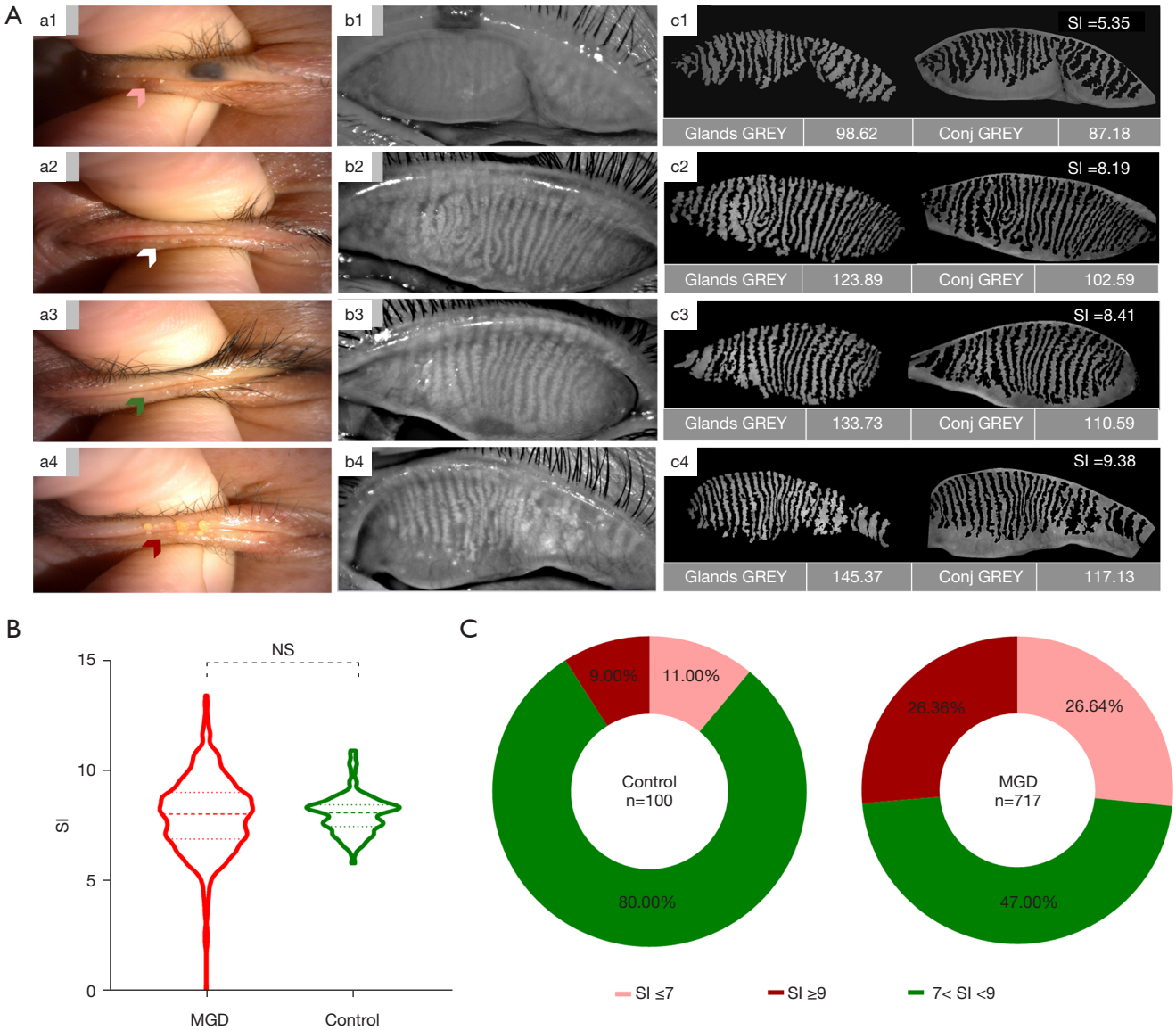

Figure 2 The participant distribution of SI and typical clinical appearances across different SI levels. Appearances of lid margins and meibum across the different SI levels. The vertical sequence 1 to 4 is arranged according to low, medium, and high SI. (A) The horizontal sequence corresponds to the appearances of lid margins and meibum. (a1) The minimal gland expression with inspissated toothpaste meibum and loss of orifices (pink red arrow) from a patient with MGD. (a2) Abundant expression of gland orifices with slightly cloudy liquid secretion (white arrow) forming in a patient with MGD. (a3) Abundant expression of gland orifices with clear liquid secretion (green arrow) from a healthy control. (a4) Abundant gland orifices expression with cloudy paste secretion and margin hyperemia (red arrow) from a patient with MGD. (b) The corresponding meibography images of sequence a. (c) The automatically segmented meibomian glands and the remaining nongland area of the everted tarsal conjunctiva area. The averaged grayscale value of the glands and nongland area in raw meibography images are shown in the tables below the figures. SI is the base 10 logarithms of the ratio of the averaged grayscale value of all meibomian glands to that of the remaining conjunctival area. (B) The distribution of SI in patients with MGD and controls. (A) GEE model was applied with the side of the eye serving as an exchangeable within-subject variable and with age and sex serving as covariates for comparisons of SI between groups at the eye levels. (C) Proportion of the three categories of SI in patients with MGD and controls. Conj, conjunctiva; SI, signal intensity; MGD, meibomian gland dysfunction; NS, not significant; GEE, generalized estimating equation.

Table 1 Cross-sectional associations between SI and clinical variables (n=817)

Parameter	β (95% CI)	P value
OSDI	0.002 (–0.004 to 0.009)	0.478
FBUT (s)	0.064 (0.028 to 0.099)	<0.001
Expressibility	–0.676 (–0.813 to –0.539)	<0.001
Meibum quality	–0.251 (–0.402 to –0.101)	<0.001
LLT (nm)	0.016 (0.009 to 0.023)	<0.001
Gland area %	0.004 (–0.023 to 0.031)	0.768

A GEE model was applied with the side of the eye serving as an exchangeable within-subject variable and with age and sex serving as covariates for the analysis of associations between SI and clinical variables. SI, signal intensity; CI, confidence interval; OSDI, ocular surface disease index; FBUT, fluorescein-stained tear-film break-up time; LLT, lipid layer thickness; GEE, generalized estimating equation.

the differences in SI, symptoms, and signs at each follow-up point were compared to those at baseline within each group. The Tukey test was used to correct for multiple comparisons. P<0.05 was taken to indicate a statistically significant difference.

Results

Cross-sectional relationships between SI and MG function

A total of 413 participants (34.23% men) with 817 eyes and an average age of 44.38 years were included in the analysis. The clinical characteristics are presented in Table S1 in the supplementary materials. After adjustments were made for age and sex, SI showed positive associations with FBUT (β=0.064) and LLT (β=0.016) and negative associations with meibum expressibility (β=–0.676) and meibum quality (β=–0.251) (all P values <0.001). Gland area ratio and OSDI did not show a significant relationship with SI (*Table 1*).

Distribution of SI levels and correlation with clinical appearances

The appearance of lid margins and meibum at different levels of SI are displayed in *Figure 2A*. The distribution range of continuous SI was wider in the MGD group (0.11 to 13.49) than in the control group (5.85 to 10.98) (*Figure 2B*). All three levels of categorical SI were observed in both the MGD and control groups. High and low SI accounted for larger proportions in the MGD group (SI ≤7: 26.64%; SI ≥9: 26.36%) than in the control group (SI ≤7: 11.00%; SI ≥9: 9.00%); while the medium SI (7< SI <9) accounted for a larger proportion in the control group (80.00%) compared to the MGD group (47.00%) (*Figure 2C*).

Participant characteristics across categories SI in patients with MGD

In the pairwise comparisons among the three categories of SI groups among patients with MGD, age, sex, and gland area ratio did not show significant differences within each pair of groups. The proportion of moderate-to-severe MGD (grade 3–5) in low SI group was the largest, while mild MGD (grade 1–2) accounted for the largest proportion in the medium SI group. The worst meibum expressibility (16% for level 3) and quality scores (19% for level 3), along with the shortest FBUT (3.82±0.13 s) and the thinnest lipid layer (65.68±2.58 nm), were observed. In the low SI group compared to the other two groups (all P values <0.05). The high SI group exhibited the worst OSDI (28.21±1.40) but the mildest meibum expressibility score (1% for level 3) and quality score (9% for level 3) compared to the other two groups (all P values <0.05). The medium SI group showed the mildest MGD severity and the mildest OSDI (26.64±1.06) compared to the other two groups (all P values <0.05) and a longer FBUT (4.79±0.13 s) as compared with the low SI group (*Table 2*).

Treatment responses in patients with MGD with different SI levels

In the longitudinal analysis, patients with high SI at baselined showed a significantly decrease in SI value after treatment at any time during the 6-month follow-up, while patients in the medium and low SI groups did not show a statistically significant difference in SI value compared to baseline at any time after LipiFlow treatment (*Figure 3*). The treatment responses according to clinical manifestations are shown in *Table 3*. Regarding the OSDI score, there were no statistically significant recovery observed in the low or high level SI group, but a significant decrease was observed across all follow-up points in the medium SI group. FBUT did not show significant recovery in the low SI group, but significant improvement was observed in the medium and high SI groups. Meibum quality was significantly improved at 15 to 90 days, but there was no statistically significant recovery at 180 days

Table 2 Participant characteristics across SI categories in the MGD group (n=717)

Parameter	SI ≤7	7< SI <9	SI ≥9	Adjusted P value		
				SI ≤7 *vs.* 7< SI <9	SI ≤7 *vs.* SI ≥9	7< SI <9 *vs.* SI ≥9
No. of eyes	191	337	189	–	–	–
Age (years), mean ± SE	48.87±1.21	46.40±0.77	41.76±0.99	0.316	0.320	0.313
Male, No. of eyes (%)	65 (34.03)	104 (30.86)	69 (36.51)	0.622	0.411	0.436
Severity (1, 2, 3, 4, 5) (%)	(2, 24, 47, 22, 5)	(28, 46, 24, 2, 0)	(24, 35, 30, 11, 0)	<0.001	<0.001	<0.001
OSDI, mean ± SE	27.99±1.41	26.64±1.06	28.21±1.40	0.001	0.013	0.022
FBUT (s), mean ± SE	3.82±0.13	4.79±0.13	4.56±0.08	<0.001	0.003	0.092
Expressibility (0, 1, 2, 3) (%)	(7, 30, 46, 16)	(18, 58, 22, 2)	(31, 53, 15, 1)	<0.001	<0.001	<0.001
Meibum quality (0, 1, 2, 3) (%)	(0, 20, 61, 19)	(0, 29, 61, 10)	(0, 41, 50, 9)	0.001	<0.001	0.001
LLT (nm), mean ± SE	65.68±2.58	72.23±2.27	80.20±2.90	0.027	0.024	0.669
Gland area ratio, mean ± SE	32.38±0.63	33.32±0.38	34.11±0.53	0.333	0.058	0.168

Differences between groups were determined via post hoc pairwise comparison testing within the generalized estimating equation analysis module with least significant difference adjustment. MGD, meibomian gland dysfunction; SI, signal intensity; SE, standard error; OSDI, ocular surface disease index; FBUT, fluorescein-stained tear-film break-up time; LLT, lipid layer thickness.

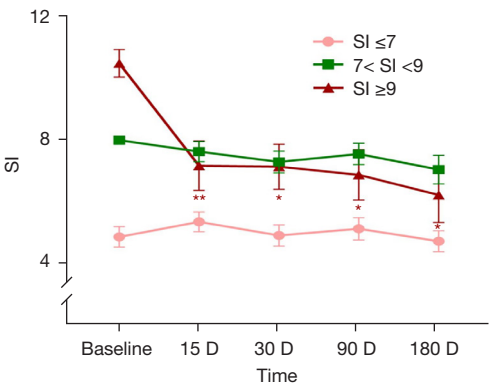

Figure 3 High signal intensity was reversed by thermal pulsation. Patients with high a SI at baseline showed a significantly decreased SI after treatment at all time points in the 6-month follow-up. *P<0.05 and **P<0.01 against the baseline. A mixed-effects model was used to identify the difference of SI at each follow-up compared with baseline within each group. The Tukey test was used to correct for multiple comparisons. SI, signal intensity; D, days.

in the low SI group. Meibum expressibility only improved at 15 and 30 days in the low SI group. There were overall improvements in meibum quality and expressibility at all follow-up points in the medium and high SI groups. Gland area ratio showed significant improvements throughout the follow-up in all three groups. LLT did not show significant differences throughout the follow-up in the three groups.

To assess the intergroup differences of the external risk factors, the proportion of eyes with long-term exposure to at least one external risk factor during the follow-up was examined, with the proportions being as follows: low SI group, 47.18%; medium SI group, 40.63%; and high SI group, 58.33%. A nonparametric test confirmed that there was no significant difference in the proportion between the groups (P=0.538). In all cases with recorded risk factor exposures, each patient reported a total of ≤1 exposure, with long-term video terminal usage being the highest proportion (82.35%). The baseline clinical characteristic of the three groups can be found in Table S2.

Discussion

In this study, we introduced an optimized automatic imaging analysis method to obtain the imaging SI from meibography, which is related to the meibum within the glands. We demonstrated significant associations between SI and the secretory activity of the MGs. Based on the clinical characteristic and treatment responses observed in the study cohort, we showed that low, medium, and high SIs can be used to determine the secretion status of the MGs.

The literature includes several subjective or semimanual methods for meibography SI measurement, but these

Table 3 Change in clinical variables after LipiFlow treatment in patients with MGD (n=78)

Group	Time (D)	OSDI		FBUT (s)		LLT (nm)		Meibum quality (%)		Expressibility (%)		Gland area ratio	
		Change	P value	Change	P value	Change	P value	Change	P value	Change	P value	Change	P value
SI ≤7 (n=34)	15	−7.73	0.08	0.88	0.72	4.71	0.63	−1.09	<0.001	−0.76	<0.001	6.44	<0.001
	30	−5.67	0.20	1.21	0.24	7.21	0.18	−0.56	<0.001	−0.50	0.002	5.91	<0.001
	90	−3.48	0.72	−0.71	0.81	0.91	>0.99	−0.56	<0.001	−0.21	0.19	6.39	<0.001
	180	−7.23	0.13	2.08	0.06	3.32	0.95	−0.12	0.61	−0.23	0.12	6.70	<0.001
7< SI <9 (n=32)	15	−10.67	0.002	2.59	<0.001	0.53	>0.99	−0.94	<0.001	−1.00	<0.001	6.12	<0.001
	30	−10.94	<0.001	2.23	<0.001	−3.78	0.77	−0.72	<0.001	−0.97	<0.001	4.11	<0.001
	90	−9.64	0.02	2.30	<0.001	−5.87	0.31	−0.91	<0.001	−0.72	<0.001	5.09	<0.001
	180	−7.78	0.04	2.70	<0.001	−8.06	0.22	−1.03	<0.001	−1.09	<0.001	4.84	<0.001
9≤ SI (n=12)	15	−6.15	0.53	3.50	0.03	0.33	>0.99	−0.92	0.03	−0.75	0.01	6.68	0.047
	30	−7.81	0.14	2.75	0.006	−6.58	0.42	−1.25	0.003	−0.92	0.03	6.38	0.029
	90	−9.38	0.30	3.75	0.005	−6.00	0.80	−1.17	0.004	−0.75	0.04	5.93	0.034
	180	−8.13	0.51	0.50	0.89	−7.83	0.73	−1.00	0.01	−0.83	0.01	6.60	0.049

The value of "change" is obtained by subtracting the baseline value from the current value. A mixed-effects model was used to identify the differences in symptoms and signs at each follow-up point as compared with the baseline within each group. The Tukey test was used to correct for multiple comparisons. MGD, meibomian gland dysfunction; D, days; OSDI, ocular surface disease index; FBUT, fluorescein-stained tear-film break-up time; LLT, lipid layer thickness; SI, signal intensity.

involve complex procedures and calculations when a large amount of images are used in clinical practice (18,19). For instances, Daniel *et al.* defined "ghost glands" as pale glands without normal gland architecture, which is a specific and subjective grading for extremely low intensity glands (18). Yeh *et al.* manually drew segmented lines using Fiji image processing software (ImageJ with plugins) along the only five central MGs to measure their pixel intensity (10). In our study, we made several improvements to the proposed tools for measuring SI in MGs. First, software was used to automatically segment the glands instead of manual segmentation, greatly improving the analysis efficiency (15,16). Second, it is known that MG function depends on the secretory activity of all the glands and not merely those located at the central eyelid (20). Therefore, all glands were included in calculating the average grayscale in this proposed software to reduce sampling error. Additionally, a grayscale filter was added for both gland and background grayscale calculations to reduce the bias in SI measurement caused by extreme grayscales value from hyperreflective light spots or eyelash shadows. Furthermore, the software implemented is user-friendly and can be operated on laptops, allowing for offline use. The analysis time for each image is within 10 seconds, and it is applicable for analyzing meibography images obtained from commercialized clinical devices. The original version of this software was validated in various disease scenarios (21,22), and this study represents the first clinical validation of measuring meibography SI to determine MG function from multicenter data with an optimized version of this software, which can pave the way for its future adoption in real-world scenarios.

The application value of SI was further confirmed through the observations in a large number of patients with MGD and a healthy control population. First, SI was found to positively correlate with meibum expressibility, meibum quality, and FBUT. Low SI was more prevalent in patients with MGD than in the control population and was associated with most severe clinical manifestations. These new findings provide evidence supporting the notion that low SI indicates low-delivery MGD, further confirming the conclusion drawn by Yeh *et al.*, who observed a reduction in MG intensity in a small sample of clinical cases of isotretinoin-induced MGD (14). Low SI tends to result in unstable tear film and lead to worse subsequent adverse effects on the ocular surface (5,9,14), potentially exacerbating dry eye and thus causing more severe MGD (23), Therefore, utmost vigilance is required for timely clinical intervention. Second, contrary to Yeh *et al.*'s

view that sufficiently high gland intensity indicates that an adequate amount of lipid is present to maintain good ocular surface health (14), we found that high level SI also showed a higher prevalence in patients with MGD than in the control population. Patients with high SI tended to exhibit excessive secretion of the viscous meibum, accompanied by lid hyperemia and cloudy lipid plugs and the highest OSDI value. These observations suggest that although the a high SI suggests active secretion of the MGs, the composition of the meibum may be unfavorably altered, leading to increased ocular surface osmolality (24) and eye discomfort, which is in line with the underlying pathology of hypersecretory MGD (25). Third, medium SI was present in the majority of healthy controls and those with mild MGD, indicating that patients with medium SI may be in the early stages of the pathological process.

The introduction of a cohort treated with LipiFlow, a standardized localized heat and pressure therapy for MGD (26), further supports the notion that different levels of meibography SI indicate varying degrees of glandular function, as there were distinct treatment outcomes between the three MGD groups categorized by SI level. By controlling for the known medical exposure factors and ensuring that there were no significant differences in external exposure factors among the groups, we could infer that the different outcomes across the three groups of varying SI was largely influenced by the treatment. In this study, the high and medium SI groups generally showed more comprehensive and long-lasting improvement compared to the low SI group, including greater meibum expressibility quality, higher OSDI, and better tear-film stability, which may be associated with a higher proportion of active acinar cell reserve (27,28). For those with low SI, the comprehensiveness and durability in efficacy of thermal pulsation therapy may be limited by an insufficient reserve of functional glands. In addition to nonpharmacological treatment, supplementation of lipid substitutes and therapies to promote gland regeneration should be considered for those with low SI. SI values have also been reported to vary between different groups within a given cohort. More specifically, patients with MGD and high baseline SI were found to experience a decrease in SI after thermal pulsation therapy (28-30). As a result, the decrease in SI in those with high SI can be attributed to the removal of pathological meibum via thermal pulsation, leading to an improvement in the quality of expressible glands (29,30). However, in our study, the medium and low SI groups, in whom meibum was either close to normal or insufficient due to a lack of expressible glands, the impact of SI via the removal of abnormal meibum was not significant (see *Figure 3*).

There were several limitations to this study which should be considered. First, we did not analyze SI in MGs from the lower eyelids due to anatomical features that prevent complete exposure of the glands; moreover, the generalizability of these results is restricted only to findings from the OCULUS Keratography 5M. Further investigation is necessary to verify whether these associations remain valid when the data are derived from other devices. Second, patients with critically severe MG dropout were not included in the treatment cohort due to insufficient glandular tissue for evaluating clinical effects. Third, controlling image quality remains a prerequisite for ensuring the usability of meibography SI. Technicians need to undergo rigorous training, and the acquisition of follow-up cohort images should ideally be performed by dedicated personnel. Furthermore, it is necessary to establish unified image quality control standards, preferably with software that can provide real-time feedback on image quality evaluation to the operators. Fourth, although we attempted to control most known external risk factors in this study, it was impossible to completely exclude the possibility of individual and environmental heterogeneity influencing the different intergroup outcomes. Future large-scale, real-world prospective studies may provide more evidence for the individualized treatment of MGD.

Conclusions

In summary, automated measurements of meibography SI can reflect glandular secretory activity. The low, medium, and high SIs can each reflect the different secretory statuses of MGD, facilitating the classification of MGD and promoting gland function-oriented treatment.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the institutional review board of Zhongshan Ophthalmic Center at Sun Yat-sen University (IRB-ZOC-SYSU; ID: 2021KYPJ215). All participating institutions were informed and agreed with the study. Informed consent was obtained from all participants prior to data collection.

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