A novel noninvasive ocular surface analyzer for the assessment of dry eye with Meibomian gland dysfunction

YUANYUAN QI[*], CHEN ZHANG[*], SHAOZHEN ZHAO, YUE HUANG and RUIBO YANG

Department of Refraction and Cornea, Tianjin Medical University Eye Hospital,
School of Optometry and Ophthalmology, Tianjin Medical University, Tianjin 300384, P.R. China

Abstract. Meibomian gland dysfunction (MGD) is a major cause of dry eye, and the purpose of the present study was to evaluate the differences between dry eye patients with MGD and controls using a novel noninvasive ocular surface analyzer. A total of 33 dry eye patients with MGD and 31 controls were enrolled in the present study. Testing included administration of the Ocular Surface Disease Index (OSDI), followed by Keratogragh 5M (Oculus, Wetzlar, Germany), measurements of the noninvasive tear film break-up times (NITBUTs), the first tear film break-up point and the morphology of meibomian gland. Meibomian gland loss (MGL) on the upper lid was evaluated using noncontact meibography. The first NITBUT (NITBUTf) was significantly shorter than the average NITBUT (NITBUTav; Mann-Whitney U-test, P<0.01). There was a weak negative Spearman correlation between NITBUTf and OSDI (rs=-0.251, P=0.046) as well as between NITBUTav and OSDI (rs=-0.250, P=0.046). Furthermore, MGL showed a statistically significant Spearman correlation with OSDI (rs=0.562; P<0.01). In the MGD group, NITBUTf was significantly shorter (P<0.01), and MGL and OSDI score were significantly greater (P<0.01 and <0.01, respectively) than in the control group. In the MGD group, the first tear film break-up point was most frequently located in the inferonasal quadrant, while it was most frequently located in the supertemporal quadrant in the control group, and there was a significant difference with this regard (χ^2=3.937, P=0.047). In the MGD group, there were significantly more breakups in the lower quadrant than in the upper quadrant (P=0.011), whereas no differences in the number of breakups in each quadrant were observed in the control group (P=0.429). Furthermore, there was no significant difference in the breakups in the lower quadrants of the two groups (χ^2=0.525, P=0.469). In the MGD group, there were significantly more breakups in the lower quadrant than in the upper quadrant (P=0.011), whereas no differences in the number of breakups in each quadrant were observed in the control group (P=0.429). Furthermore, there was no significant difference in the breakups in the lower quadrants of the two groups (χ^2=0.525, P=0.469). As the NITBUTs showed only a weak correlation with OSDI, they may not be a strong indicator of the OSDI. However, MGL indicated changes of meibomian gland morphology in the MGD group and the first tear film break-up point was likely located in the inferonasal quadrant in the MGD group.

Introduction

Dry eye has become a common ocular surface disease that affects patients' quality of life (1). In recent years, the incidence of dry eye has gradually increased in China. At present, invasive testing methods for this disorder are widespread in the process of clinical diagnosis and treatment. They are used to evaluate the stability of the tear film, the quantity of secretion and the extent of damage to the ocular surface. However, these testing methods require direct contact with the ocular surface, which can cause discomfort of the eyes. In addition, these diagnostic methods are often not properly performed, leading to contradictory results (2,3). During the process of diagnosis, the final result is at times dependent on the subjective judgment of the examiner (4). In the present study, noninvasive tests to overcome these problems were characterized with the aim of providing data to support the use of this method as a more objective means of tear film assessment and diagnosis of dry eye.

Meibomian glands (MGs) secrete lipids into tears, forming a superficial lipid layer that stabilizes the tear film (5). MG dysfunction (MGD) is a major cause of dry eye, resulting from an abnormal quality or quantity of the lipid layer that leads to an unstable tear film and increased tearfilm osmolarity (6).

The cornea is easily damaged if the superficial tear film that covers it is disrupted, and at present, numerous tests are available for measuring this tear film. Apart from invasive testing methods, certain advanced noninvasive tests, including the video-meniscometer, the interferometer and laser meibometry,

Correspondence to: Dr Shaozhen Zhao, Department of Refraction and Cornea, Tianjin Medical University Eye Hospital, School of Optometry and Ophthalmology, Tianjin Medical University, 251 Fukang Road, Nankai, Tianjin 300384, P.R. China
E-mail: zhaosz1997@126.com

[*]Contributed equally

are being used to characterize the pathophysiological aspects of dry eye by measuring the tear meniscus radius, analyzing the tear lipid layer and quantifying the amount of meibomian lipid on the lid margin, respectively (7). In addition, the Tearscope (Keeler Ltd., Windsor, UK) and the videokeratoscope (4,8) noninvasively measure the noninvasive tear break-up time (NITBUT). These techniques do not require fluorescein and do not alter the volume or the properties of the tear layer.

Quantitative evaluation of MG morphology is important in assessing the severity of MGD and the effects of therapeutic interventions. Numerous studies on MG morphology are currently available (9-11), but studies investigating the location of the first tear film break-up point are relatively rare (12). An examiner-independent device, the Keratograph 5M, was introduced for clinical use in 2013 in China. It allows for detection of the NITBUT using automation with a larger field of observation than the Tearscope (4). It largely eliminated operator bias compared with other devices (4). In addition, screening tests demonstrated that it was able to exactly record the first location of the tear film break-up point and easily observe changes of MG morphology (13,14).

In the present study, the Keratograph 5M was therefore selected to evaluate differences between the MGD and control groups by detecting the NITBUT, changes of MG morphology and the location of the first tear break-up point.

Materials and methods

Subjects. The present study was of a prospective, observational nature. It included 33 dry eye patients with MGD who were diagnosed at the Tianjin Medical University Eye Hospital (Tianjin, China) between November 2013 and March 2014, as well as 31 control subjects. The right eye from each subject was underwent further examination. Written informed consent was obtained from all subjects and the study was performed in accordance with the tenets of the Declaration of Helsinki. Ethical approval was obtained from the Ethics committee of Tianjin Medical University (Tianjin, China).

The MGD group was comprised of subjects with clinical symptoms and/or signs of dry eye, clinical signs of MGD, terminal duct obstructions, clogging of the MG, turbid secretions or turbid secretions with clumps, inflammation and swelling of the eyelid margin, posterior lid margin hyperemia or symptoms of MGD for at least 3 months (15). The control group did not have any clinical signs and/or symptoms of MGD, posterior blepharitis, dry eye, or significant ocular surface disease, and were not using eye drops or eye medications. Exclusion criteria included the following: i) Age of ≥18 or <30 years ii) systemic diseases affecting the ocular surface, iii) any disease affecting the ocular surface (e.g., acute conjunctivitis, blepharitis, meibomianitis or any anterior segment disease), iv) previous ocular surgery or trauma, v) use of contact lenses, vi) use of any medication affecting the ocular surface (e.g., artificial tears, neostigmine or atropine) for ~6 months and vii) a common cold within two weeks.

Study protocol

Ocular Surface Disease Index (OSDI). Each subject's symptoms were evaluated by a validated Chinese translation of the OSDI questionnaire (10,16,17). Total OSDI scores were calculated as recommended by Schiffman *et al* (17).

NITBUT. NITBUT measurements were obtained from the right eye using the Keratograph 5M (OCULUS Optikgeräte GmbH, Wetzlar, Germany) in accordance with the manufacturer's instructions. After manual focusing, the subjects were asked to blink twice and then to keep their eyes open as long as possible. Each recording was discontinued at the next eye blink (to avoid reflex tearing, subjects were encouraged to blink if they felt discomfort). A single examiner collected the data. The first result was recorded. Measurements were repeated if the initial measurement could not be obtained or if tears were shed in response to an irritation on the first attempt. The first tear break-up time was noted as the first NITBUT (NITBUTf) and the average value of the intervals after blinking across the observed area on the cornea were documented as the average NITBUT (NITBUTav). All patients were tested in the same room.

First tear film break-up point. Patients were requested to open their eyes as long as possible when measuring the NITBUTs. When irregular roundness or cracked roundness in the placido disc appeared at the first observation, the corresponding site was noted as the first tear film break-up point and the simultaneous time indicated the NITBUTf. The Keratograph 5M clearly displayed different tear film break-up points in different colors and the first break-up point of ≤6 sec was usually expressed in red. If the tear film break-up time was longer, the corresponding color changed from red to green in a heat map-like fashion (Fig. 1).

With the center of the pupil as the boundary, the tear film average was divided into four parts involving the supernasal, inferonasal, supertemporal and infratemporal quadrants.

MG loss (MGL). Images showing MGL of the right upper eyelid were obtained using the Keratograph 5M in accordance with the manufacturer's instructions and analyzed using the polygon selection tool of ImageJ software (version 1.47v/Java 1.6.0_20; National Institutes of Health, Bethesda, MD, USA; Fig. 2) (10). The ratio of meibomian gland dropout area to the total area was recorded.

Statistical analysis. Values are expressed as the mean ± standard deviation. Differences between each measurement in the MGD group and the control group were evaluated using the Mann-Whitney U-test. Shapiro-Wilk testing of the data resulted in OSDI (P<0.01), NITBUTf (P<0.01), NITBUTav (P<0.01) and MGL (P<0.05), indicating the samples were not normally distributed. These findings were consistent with histogram, notched-box and normal quantile plots. The decision to use Spearman correlation was then made. The Cochran-Mantel-Haenszel χ^2 test was used to analyze differences in parts of the first tear film break-up point located between the two groups. The Binomial test was used to analyze differences within the same group regarding the first tear film break-up point. Statistical significance was set at P≤0.05. Statistical analysis was performed using SPSS 19.0 software (IBM SPSS, Armonk, NY, USA).
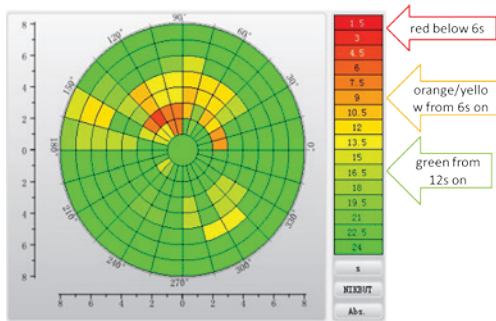
Figure 1. Output of different tear film break-up points measured by Keratograph 5M (software version 2.7r12; Oculus, Wetzlar, Germany). The progress of tear film break-up was plotted as a tear-map. The colored tear-map clearly shows the locations of tear film break-up point at different time points. Red, 0-6 sec; orange/yellow, 6-12 sec; green, 12-24 sec.
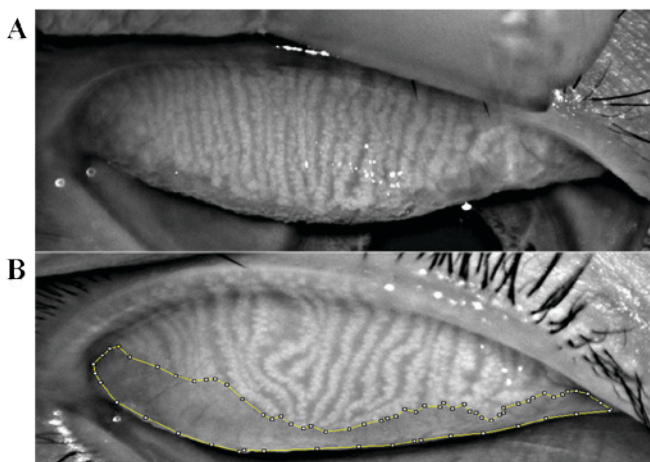

Figure 2. (A) Normal Meibomian gland morphology with almost no missing areas. (B) Analysis of the ratio of meibomian gland dropout area to the total area using Image J software.

Figure 3. Association between NITBUTf and OSDI, indicating a slight negative Speaman correlation (rs=-0.251; P=0.046). OSDI, Ocular Surface Disease Index; NITBUTf, first noninvasive tear film break-up time.

Figure 4. Association between NITBUTav and OSDI, indicating a slight negative Speaman correlation (rs=-0.250; P=0.046). OSDI, Ocular Surface Disease Index; NITBUTav, average noninvasive tear film break-up time.

Results

Patient characteristics. A total of 64 subjects were included in the present study, comprising the MGD group and the control group. The MGD group included 33 subjects (18 males and 15 females) and the control group included 31 subjects (15 Males and 16 females). The mean age in the MGD group was 24.2±1.63 years (range, 21-28 years) and that of the control group was 24.1±1.98 years (range, 21-29 years). Differences in age or gender between the MGD and the control were not statistically significant (χ^2=0.063, P=0.803).

NITBUTf and NITBUTav show a light negative, while MGL shows a positive correlation with OSDI. Spearman correlation analysis was performed to analyze the correlations between OSDI and NITBUTf (rs=-0.251; P=0.046; Fig. 3), OSDI and NITBUTav (rs=-0.250; P=0.046; Fig. 4), and OSDI and MGL (rs=0.562; P<0.01; Fig. 5). The NITBUTf was significantly shorter than the NITBUTav according to the Mann-Whitney U-test (P<0.01), and NITBUTf and NITBUTav decreased as OSDI increased in all participants, although the two negative correlations were weak. MGL and OSDI exhibited a positive correlation. Mann-Whitney U-tests were also used
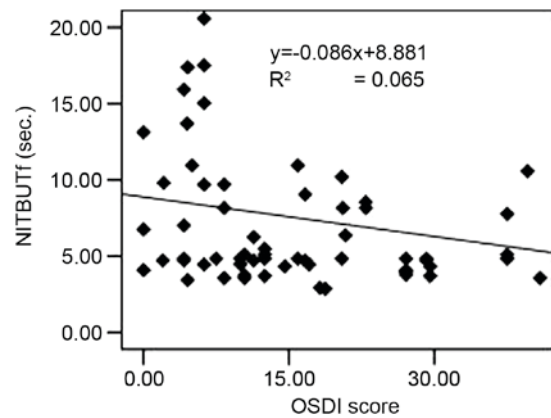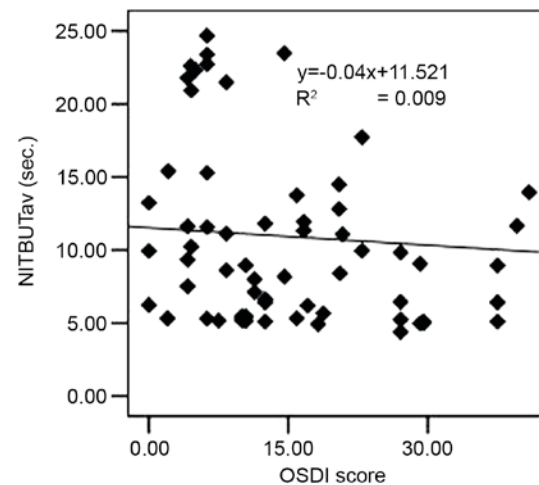
to analyze the data between the MGD group and the control group. The NITBUTf and NITBUTav in the MGD group were shorter than the control group (P=0.015 and P=0.033, respectively). The percentages of MGL and OSDI in the MGD group were greater than those in the control group (P<0.01 for each; Table I).

Location of the tear break-up point differs in patients with MGD group but not in controls. The location of the first tear film break-up point was detected in different quadrants. In the MGD group, the first tear film break-up point of 7 eyes (21.2%) was located in the supernasal quadrant, that of 12 eyes (36.4%) in the inferonasal quadrant, that of 6 eyes (18.2%) in the supertemporal quadrant and that of 8 eyes (24.2%) was located in the infratemporal quadrant. However, in the control group, the first tear film break-up point in the aforementioned quadrants was in 6 eyes (19.4%), 8 eyes (25.8%), 9 eyes (29.0%) and 8 eyes (25.8%), respectively. The MGD group had a higher proportion of first tear film break-up points in the inferonasal quadrant than the normal controls and there was a significant difference between the two groups (Cochran-Mantel-Haenszel

Table I. Comparison of clinical characteristics between MGD group and control group.

Parameter	MGD group (n=33)	Control group (n=31)	P-value
Age (years)	24.21±1.63	24.13±1.98	0.064
Gender (n)			0.803
Male	18	15	
Female	15	16	
OSDI	22.54±17.04	10.39±6.57	0.002
NITBUTf (sec)	5.69±2.65	9.30±5.90	0.015
NITBUTav (sec)	9.32±4.64	12.51±6.76	0.033
MGL (%)	38.22±10.02	20.08±10.10	<0.001

MGD, Meibomian gland dysfunction; MGL, Meibomian gland loss; OSDI, Ocular Surface Disease Index; NITBUTf, first noninvasive tear film break-up time; NITBUTav, average NITBUT.
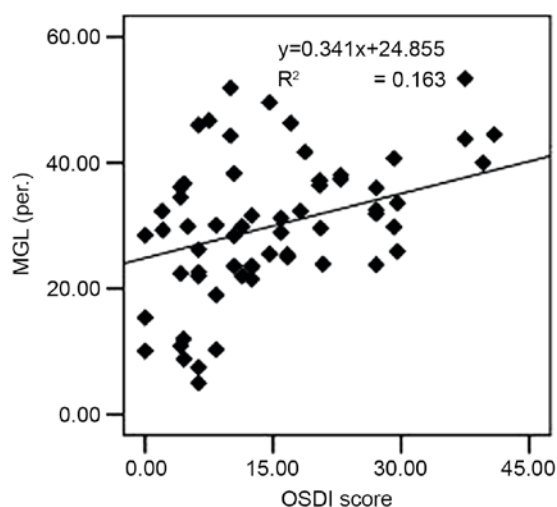

Figure 5. Association between MGL and OSDI, indicating a positive Speaman correlation (rs=0.562; P<0.01). OSDI, Ocular Surface Disease Index; MGL, Meibomian gland loss.

χ^2 test, χ^2=3.937, P=0.047). In addition, the supernasal quadrant and the supertemporal quadrant were classified as a new quadrant (upper quadrant), while the inferonasal quadrant and the infratemporal quadrant were combined and classified as the lower quadrant. Using these classifications in the MGD group, the first tear film break-up point was located in the upper quadrant in 13 eyes (39.4%) and in the lower quadrant in 20 eyes (60.6%). In the control group, the first tear film break-up point in 15 eyes (48.4%) was located in the upper quadrant and in 16 eyes (51.6%) in the lower quadrant (Table II). For the MGD group, there were significantly more breakups in the lower quadrant than in the upper quadrant (Binomial Test, P=0.011). However in the control group, the percentage of breakups observed appeared to be ~50% in the lower and upper parts, with no significant differences between them (Binomial Test, P=0.429). Although the percentage in the lower quadrant in the MGD group was higher than that in the control group, there was no significant difference

between the two groups (Cochran-Mantel-Haenszel χ^2 test, χ^2= 0.525, P=0.469).

Discussion

Dry eye is a multifactorial disease of the tears and ocular surface resulting in symptoms of discomfort, visual disturbance and tear film instability, which can potentially damage the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface (18). MGD, which can eventually lead to dry eye, is a chronic and diffuse abnormality of the MG, commonly characterized by terminal duct obstructions or excessive keratosis and/or quantitative/qualitative changes in glandular secretion and/or lipid discharge difficulty (19). This situation may result in alterations to the tear film, increased tearfilm osmolarity, symptoms of eye irritation, apparent eye inflammation and ocular surface disease (19). These problems have prompted researchers to identify novel methods to diagnose, monitor and treat MGD in a timely manner.

The present study used the Keratograph 5M to characterize noninvasive parameters and to observe differences between the MGD and control groups. The light wave and energy of the instrument did not exceed the safety margin for the eye; operation of the device did not cause instrument-induced reflex tearing or characterize phenomena interfering with the tear film. However, the instrument was equipped with an infrared light source allowing for observation of the NITBUT and changes of MG morphology, as well as determining the location of the tear break-up point using tear film reflection.

A total of 33 patients with dry eyes and MGD and 31 control subjects were recruited for the current study. All subjects were characterized using the Keratograph 5M. There were significant differences in NITBUTs, OSDI and MGL between the MGD group and the control group. These findings were similar to those of previous studies (20,21). In the present study, the NITBUTs in the MGD group were shorter than those in the control group, indicating that MGD affected the stability of the tear film. When the stability was disturbed, evaporation of the tear film increased. MGL was greater in the MGD group, possibly indicating that the morphology and function of the MG was altered in MGD. The OSDI was significantly greater in the MGD group, possibly due to ocular surface damage occurring due to changes in MG morphology and function, and due to instability of the tear film. Spearman correlation analysis revealed a weak negative correlation between NITBUTs and OSDI (P=0.046 and 0.046, respectively). These results are similar to those reported by Fuller *et al* (4), who used the Keratograph 4. They were also similar to those reported by Best *et al* (22); however, the current study identified a lower correlation with the OSDI than that obtained using the Tearscope.

A number of possible factors may account for these differences. Firstly, in the present study, there were no significant differences in age or gender between the two groups, as indicated by the results of the Mann-Whitney U-test (P=0.803). However, the exclusive enrolment of subjects aged ≤30 may have affected the outcome, as dry eye is more severe and more prevalent in the older population. Secondly, adherence to

Table II. Descriptive statistics of the different locations of the first tear film break-up point.

Group	Supernasal, n (%)	Supertemporal, n (%)	Inferonasal, n (%)	Infratemporal, n (%)	Total, n (%)
MGD	7 (21.2)	6 (18.2)	12 (36.4)	8 (24.2)	33 (100)
		13 (39.4)[a]		20 (60.6)[b]	
Control	6 (19.4)	9 (29.0)	8 (25.8)	8 (25.8)	31 (100)
		15 (48.4)[a]		16 (51.68)[b]	

MGD, Meibomian gland dysfunction. [a]Upper eye quadrant values; [b]Lower eye quadrant values.

the manufacturer's guidelines for NITBUT testing may have induced a sampling bias, as validation studies suggested that repeated sampling three times and determination of the average value improved reliability (23). Tear debris may have created a premature tear film break-up that led to lower NITBUT values. Without repeated measures, certain instrumental bias may have resulted from the influence of tear film debris artifacts on the NITBUT values. The instruments used may have been another important factor. Compared with the Tearscope, the Keratograph 5M has certain advantages, including a constant contrast independent of iris color, a larger field of observation and a greater elimination of operator bias (23,24). The Keratograph 5M also has several advantages compared with the Keratograph 4, such as replacement of the white light-emitting diode sources with infrared diodes, to greatly decrease the instrument-induced reflex tearing (4). However, as with other objective assessments, the software of included with the Keratograph 5M had a default threshold involving sensitivity to the size of the break-up area, which may have led to an internal bias. However, using the software to interpret a tear break is unlikely to be adjusted in clinical practice and this potential internal bias should therefore not be inconsistent among subjects or visits, as it had no effect on the diagnosis of tear film instability. The above reasons may explain why a lower correlation between NITBUTs and OSDI was detected in the present study compared with previous studies that used the Tearscope (4,22).

Pult and Reide (10) found that OSDI and MGL had a positive association and the present study came to the same conclusion. Notably, MGL may be a parameter to diagnose symptoms of dry eye. It has been reported that an MGL of >32% is likely to be accompanied with associated, detectable clinical symptoms (10). For clinicians, determination of changes in MG morphology, the percentage of MGL and the different of NITBUTs, including NITBUTf and NITBUTav, can be a painless and noninvasive method for diagnosing dry eye, which may assist in early treatment and prevention of further aggravation.

The lipid layer can prevent water evaporation from the ocular surface and stabilize the tear film by reducing the tension on the surface between the gas and liquid. The lipid layer thickness and the question whether it distributes quickly and evenly on the ocular surface following scardamyxis can have a significant role in stabilization of the tear film (25). MGD features an abnormal lipid layer in quantity or quality, which may lead

to reduced tear film stability and breaking, which then affects the thickness of the entire ocular tear film. The objective of the present study was to visually observe the location of the thinnest tear film point in patients with MGD and control subjects using the Keratograph 5M. The results demonstrated that the first tear film break-up point was more frequently located in the lower part in the MGD group (P=0.011). In the control group, however, the two percentages appeared to be ~50% (P=0.429). Furthermore, there was no significant difference in the first tear film break-up points between the two groups (P=0.469). A fluorophotometric study by Benedetto et al (26) implied that inferior tear film was potentially thinner than the superior in normal individuals. Zhuang et al (27) used interferometry to measure tear film thickness at different points along the corneal vertical meridian and observed the same phenomenon documented by Benedetto et al. In the present research, the result of the MGD group was consistent with previous studies but the results for the control group differed. This may be due to factors, such as interindividual variability, technology bias or sample size, leading to an inconsistent result. However, tear film thickness not only depends on the lipid layer but also relies on tear volume, tear surface tension, tear viscosity and upper lid speed. All of these factors independently vary between subjects (28) and previous studies have also reported a considerable inter-individual variability in tear film thickness (12,29). Zhuang et al (27) even reported thickness variations in the vertical direction. In view of tear film spatial variations, they may be classified into three patterns in normal as well as in dry eyes: Thickening upward, uniform distribution and thickening downward. In the present study, the ocular surface was divided into four quadrants: Inferonasal, supernasal, supertemporal and infratemporal. However, the MGD group had a significantly higher proportion of first tear film break-up points on the inferonasal part than the control group (P=0.047). These findings differed from the results of a previous study (14), which reported that the first tear film break-up point was most frequently located in the inferonasal quadrant in the dry eye and control groups, with no significant differences between the two groups. It is possible that the interindividual variability in tear film thickness accounts for this difference. Other factors, such as age, may have affected the results, suggesting that future studies using different age groups are required. In addition, instrument bias was present. The aforementioned study used the Keratograph 4 (14), which potentially resulted in more reflex tearing that may have

affected the distribution of the tear film over the ocular surface more than the Keratograph 5M.

In addition, the current study had certain shortcomings regarding its design: Firstly, although there was no significant difference in gender, a relatively narrow age range was covered, with only relatively young subjects included. This was because the current study included those willing to undergo LASIK surgery, who were mostly <30 years old. Age can affect the severity of dry eye: The incidence of dry eye increases with age and becomes more severe. Therefore, a larger study including a wider age range should be performed in the future. However, the present study was able to characterize differences between the two groups at a young age. Second, the control group may have contained subjects with non-obvious MGD. Clinical MGD grading was not considered in the present analysis. In further studies, patients may require classification in more detail in order to determine whether noninvasive assessment can provide further information.

In conclusion, the current study demonstrated that NITBUTs were not a strong indicator of OSDI. MGL exhibited changes of meibomian gland porphology in the MGD group and location of the first tear film break-up point was more likely to occur in the inferonasal quadrant in MGD group. The current study determined that the Keratograph 5M could be feasibly used to observe the tear film break-up time and point, as well as the morphology of the meibomian gland.

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