Analysis of Factors Associated With the Tear Film Lipid Layer Thickness in Normal Eyes and Patients With Dry Eye Syndrome

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Purpose. To determine the effects of clinical variables, including age, sex, history of refractive or cataract surgery, contact lens use, and ocular surface and meibomian gland parameters on the lipid layer thickness (LLT) in normal subjects and patients with dry eye syndrome (DES).

Methods. A total of 64 normal subjects and 326 patients with DES were enrolled, and they underwent measurements of LLT with a LipiView interferometer and tear meniscus height using optical coherence tomography, tear film break-up time (TBUT) determination, ocular surface staining, Schirmer's test, examination of the lid margins and meibomian glands, and assessment using the Ocular Surface Disease Index (OSDI).

Results. In normal subjects, the median (range) LLT was 67 (33–100) nm, and age was the only factor that was significantly associated with LLT ($\beta = 0.678$, $P = 0.028$). In patients with DES, the median (range) LLT was 84 (20–100) nm, and 79.0% of the participants fulfilled the diagnostic criteria for meibomian gland dysfunction (MGD). In a multivariate analysis, increased age and female sex were significantly related to increased LLT ($\beta = 0.282$, $P = 0.005$ and $\beta = 11.493$, $P < 0.001$), and hypersecretory MGD and lid margin inflammation were independently associated with increased LLT ($\beta = 11.299$, $P = 0.001$ and $\beta = 12.747$, $P = 0.001$).

Conclusions. Lipid layer thickness measurements using a new interferometer are significantly affected by demographic factors such as age, sex, ocular surgical history, and MGD type. Therefore, all of these factors must be considered in the diagnosis of ocular surface diseases.

Keywords: lipid layer thickness, dry eye syndrome, meibomian gland dysfunction

Tear film instability is known as one of the core mechanisms of dry eye syndrome (DES).[1] The lipid layer stabilizes the tear film and prevents tear evaporation from the aqueous tear film layer.[2] These characteristics of the lipid layer can be evaluated based on its thickness and on its structure and composition.[3]

The International Workshop on Meibomian Gland Dysfunction (MGD) has defined MGD as a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion.[4] Therefore, lipid layer thickness (LLT) may be a marker of changes in meibum secretion, and LLT measurement is expected to be helpful for the assessment and classification of MGD (obstructive or hypersecretory MGD).

Several studies showed a positive correlation between the LLT measurement and expressible meibomian glands and suggested a higher probability of obstructive MGD in patients with a low LLT.[5–9] One study reported that an LLT of less than or equal to 75 nm could be used for the detection of obstructive MGD (sensitivity of 65.8% and specificity of 63.4%).[6] Another study demonstrated that the LLT correlated with meibomian gland loss using noncontact meibography in the obstructive MGD group.[5] However, despite previous reports on the clinical significance of a lower LLT, we occasionally encounter discrepancies between the LLT value and the ocular findings, including much lower LLT values in normal subjects or higher LLT values in patients with severe MGD.

We hypothesized that the results of LLT measurements may be affected by various demographic or ocular factors other than the meibomian gland status. The purpose of our study was to determine the effects of age, sex, history of ocular surgery and contact lens use, and ocular surface and meibomian gland parameters on LLT measurements obtained using a LipiView interferometer (TearScience Inc., Morrisville, NC, USA). We did not attempt to compare clinical features between normal subjects and patients with DES; however, we evaluated the effects of clinical variables on LLT measurements within each group.

Methods

Subjects

This study followed the tenets of the Declaration of Helsinki, and the prospective study protocol was approved by the Severance Hospital institutional review board, Seoul, South Korea (No. 4-2015-1009) and registered at ClinicalTrials.gov (identification number: NCT02645045). Informed consent was obtained from all patients after an explanation of the purpose and possible consequences of the study.

All subjects in this study were of the same ethnicity: Koreans aged 20 to 80 years. Normal subjects were recruited among

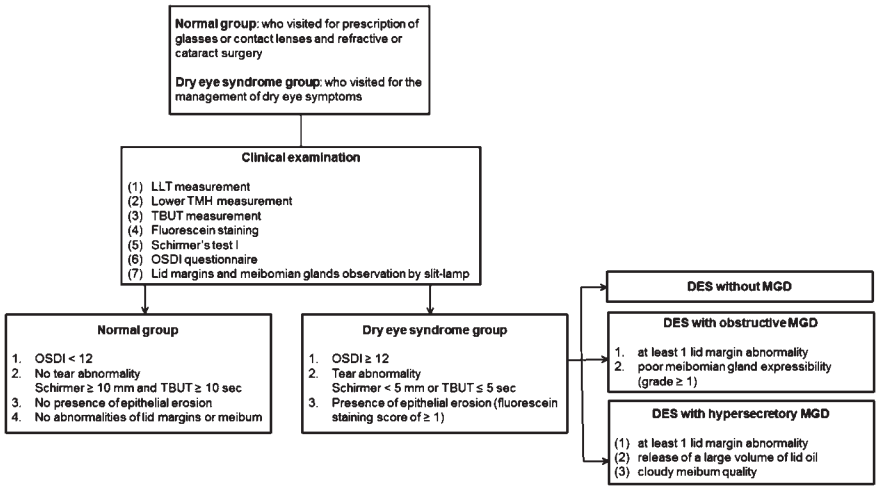

FIGURE 1. Flowchart showing subjects' progress throughout the study.

outpatients who visited for prescription of glasses or contact lenses and refractive or cataract surgery, and patients with DES were consecutively recruited among outpatients who visited for the management of dry eye symptoms from December 2015 to February 2016 in the Department of Ophthalmology at Severance Hospital. We excluded patients of less than 20 years of age; those with histories of ocular surgery within 3 months, ocular injury, or ocular diseases such as ocular infection, allergy, and autoimmune disease; and those using a punctal plug or topical ocular medications other than artificial tears. Patients who used artificial tears were instructed not to apply them for at least 12 hours before the examinations. According to the diagnostic criteria given below, the candidate subjects were classified into two groups: normal ($n = 64$) and DES ($n = 323$).

Outcome Measures

Clinical examinations were performed by one of the authors (JWJ), and data were obtained from the right eye unless right eye was excluded from the study, in which case ($n = 2$ in normal group, $n = 10$ in DES group) data were collected from the left eye.

All measurements were performed sequentially as follows (Fig. 1): (1) LLT measurement was conducted using a LipiView interferometer as previously described.[5] The measurement area was set with the pupil placed in the center of the live video screen and the green targeting rectangle 1 mm above the inferior tear meniscus. The camera focus was then manually adjusted for the clear interferometry image of the tear film. While the participant maintained a fixation on the internal target, images were captured. The participants were allowed to blink naturally during image capture. The LLT is presented in interferometric color units (ICU), where 1 ICU corresponds to approximately 1 nm. We used a LipiView II interferometer, which displays a maximum of 100 nm in any case with an LLT of greater than 100 nm LLT, (2) the lower tear meniscus height (TMH) was evaluated using Fourier-domain optical coherence tomography (FD-OCT; RTVue; Optovue, Inc., Fremont, CA, USA) 5 minutes after LLT measurement, as previously reported.[10] Vertical 2-mm scan images at the middle of the lower eyelid were obtained two times per eye, and the TMH was measured using virtual calipers in the FD-OCT software. Tear meniscus height was defined as the distance between the upper meniscus on the cornea and the lower meniscus on the lid, (3) tear film break-up time (TBUT) was measured by applying a single fluorescein strip (Haag-Streit, Koeniz, Switzerland) to the inferior palpebral conjunctiva after instilling a drop of normal saline. The mean time for three attempts was recorded, (4) after measuring the TBUT, corneal and conjunctival staining was graded from 0 to 5 according to the Oxford staining score based on the pattern of fluorescein staining notes on slit-lamp biomicroscopy,[11] (5) Schirmer's test I was performed without topical anesthesia by placing a Schirmer strip in the midlateral portion of the lower fornix. The amount of wetting was recorded after 5 minutes, and patients were asked to keep their eyes lightly closed during the test, (6) subjective symptoms were graded on a numerical scale from 0 to 4, according to the validated 12-item Ocular Surface Disease Index (OSDI) questionnaire. The total OSDI was calculated using the following formula: OSDI = (sum of scores for all questions answered × 100)/(total number of answered questions × 4), which ranges from 0 to 100,[12] and (7) the lid margins and meibomian glands were checked for lid margin abnormalities, gland expression, and meibum quality, as previously described.[4,10,13–15] Lid margin abnormalities were scored as 0 (absent) or 1 (present) for the following parameters: vascular engorgement, plugged meibomian gland orifices, anterior or posterior displacement of the mucocutaneous junction, and irregularity of the lid margin.[4,10,13,14] The presence of an inflamed lid margin was checked. The degree of meibomian gland expressibility using firm digital pressure applied on five glands of the central third of the lower lid was graded as follows: grade 0, all five glands expressible; grade 1, three to four glands expressible; grade 2, one to two glands expressible; and grade 3, no glands expressible.[13,15] The meibum quality over eight lower lid glands was graded as follows: grade 0, clear; grade 1, cloudy; grade 2, cloudy with granular debris; and grade 3, thick, like toothpaste. Each of the eight glands of the lower eyelid was graded on a scale from 0 to 3. The scores of the eight glands were summed to obtain a total score (range, 0–24).[10,13]

The MGD grade was determined based on the three lid parameters: MGD grade 1, minimally altered expressibility (grade 1) and secretion quality (grade ≥ 2, <4); MGD grade 2, scattered lid margin features, mildly altered expressibility (grade 1) and secretion quality (grade ≥ 4, <8); MGD grade 3, lid margin features of plugging, vascularity, moderately altered expressibility (grade 2) and secretion quality (grade ≥ 8, <13); MGD grade 4, lid margin features of dropout,

Table 1. Univariate and Multivariate Linear Regression Analysis to Evaluate the Impact of Clinical Variables on Lipid Layer Thickness in Normal Group

Variables	Normal Eyes (64 Eyes From 64 Individuals)	Univariate Model		Multivariate Model	
		Beta (SE)	*P* Value	Beta (SE)	*P* Value
Age, y; median (range)	32 (20–67)	0.261 (0.331)	0.430	**0.678 (0.309)**	**0.028**
Sex, *n* (%)					
Male	42 (65.6)	1 (Ref)		1 (Ref)	
Female	22 (34.4)	11.651 (8.149)	0.153	10.357 (8.062)	0.199
Ocular history, *n* (%)					
No history	51 (79.7)	1 (Ref)		1 (Ref)	
Refractive surgery	5 (7.8)	21.539 (14.121)	0.127	6.155 (13.144)	0.640
Cataract surgery	5 (7.8)	−7.462 (10.352)	0.508	−21.974 (11.005)	0.056
Contact lens use	3 (4.7)	38.120 (19.413)	0.060	28.135 (17.904)	0.129
Ocular surface parameters, median (range)					
Lipid layer thickness, nm	67 (33–100)				
Subjective score (OSDI)	4.20 (0.00–10.00)	−0.616 (0.736)	0.403		
Schirmer's test I value, mm	18 (10–27)	0.064 (0.615)	0.918		
TBUT, s	10 (10–25)	0.722 (0.752)	0.337		
Corneal staining score (0–5)*	0 (0–0)	−0.476 (15.862)	0.976		
Conjunctival staining score (0–10)*	0 (0–0)	8.442 (6.661)	0.205		
TMT, μm by FD-OCT	316 (249–370)	−0.011 (0.053)	0.833		

Bold numbers indicate statistically significant results.
* Oxford staining score.

displacement, severely altered expressibility (grade 3), and secretion quality (grade $\geq$ 13).[4]

The criteria for the normal group were as follows: (1) OSDI of less than 12, (2) no tear film abnormality (Schirmer's test value of $\geq$10 mm after 5 minutes and TBUT of $\geq$10 seconds), (3) no presence of corneal or conjunctival epithelial erosion as evidenced by a fluorescein staining, and (4) no abnormalities of the lid margins or meibum. Dry eye syndrome group included patients who fulfilled the diagnostic criteria as follows[16]: (1) the presence of dry eye symptoms (OSDI $\geq$ 12), (2) abnormal tear production as determined by Schirmer's test I (<5 mm) or abnormal tear film stability as determined by the TBUT (<5 seconds), and (3) the presence of corneal or conjunctival epithelial damage as evidenced by a fluorescein staining score of greater than or equal to 1, based on the Oxford score. MGD is defined as a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion.[4] The subtype of MGD was classified into two categories according to secretion; low-delivery states as obstructive MGD and high-delivery states as hypersecretory MGD.[4] The obstructive MGD subgroup included patients who fulfilled the following diagnostic criteria[16]: (1) at least one lid margin abnormality, and (2) poor meibomian gland expressibility (grade $\geq$ 1) on examination of the lid margin and meibomian gland by one examiner. The hypersecretory MGD subgroup included patients who fulfilled the diagnostic criteria[17,18]: (1) at least one lid margin abnormality, (2) release of a large volume of meibum at the lid margin in response to expression, and (3) cloudy meibum quality. As a result, DESs were classified into three subgroups; DES without MGD, DES with obstructive MGD, and DES with hypersecretory MGD.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 20.0; SPSS Inc., Chicago, IL, USA). As the majority of the variables did not have a normal distribution, nonparametric tests were adopted. Analyses included the frequency for categorical data and the median (range) for continuous data. Fisher's exact test was used to compare categorical variables, and the Kruskal-Wallis test was used to compare the groups for numeric variables. Univariate and multivariate linear regression analyses were computed to evaluate the impact of clinical variables on LLT in the normal group and the DES group, respectively. For the multiple linear regression analysis, 20 subjects per independent variable are recommended.[19] Based on this reference; we determined the sample size of the DES group by considering 15 possible independent variables. *P* values less than 0.05 were considered significant.

Results

Characteristics and Factors Associated With Lipid Layer Thickness in the Normal Group

The median age was 32-years old, and 34.4% of the participants in this group were women. The percentages of subjects with a history of refractive surgery, cataract surgery, and contact lens use were 7.8%, 7.8%, and 4.7%, respectively. The ocular surface parameters, including the OSDI scores, Schirmer's test I scores, TBUT, ocular surface staining scores, and TMH, are presented in Table 1. In the normal group, the median (range) LLT was 67 (33–100) nm. On multivariate analysis, age was found to be an independent factor of LLT ($\beta = 0.678$, $P = 0.028$). No other variables showed a significant impact on LLT in the normal group.

Characteristics and Ocular Surface Status in the Dry Eye Syndrome Group

The median age was 54-years old, and 73.4% of the participants in this group were women. Of 323 subjects, 43 (13.3%) had undergone refractive surgery, 42 (13.0%) had undergone cataract surgery, and 10 (3.1%) used contact lenses. Table 2 shows the ocular surface parameters in the DES group, and the

Table 2. Clinical Variables and Ocular Surface Status in Dry Eye Syndrome Group

Variables	DES With/Without MGD ($n = 323$)	DES Without MGD ($n = 68$)	DES With Obstructive MGD ($n = 204$)	DES With Hypersecretory MGD ($n = 51$)	*P* Value*
Age, y	54 (20–80)	45 (23–72)	54 (20–80)	59 (20–80)	**0.048**
Sex, *n* (%)					0.901†
Male	86 (26.6)	19 (27.9)	54 (26.5)	13 (25.5)	
Female	237 (73.4)	49 (72.1)	150 (73.5)	38 (74.5)	
Ocular history, *n* (%)					**0.039†**
No history	228 (70.6)	43 (63.2)	146 (71.6)	39 (75.9)	
Refractive surgery	43 (13.3)	13 (19.1)	28 (13.7)	2 (3.7)	
Cataract surgery	42 (13.0)	8 (11.8)	24 (11.8)	10 (20.4)	
Contact lens use	10 (3.1)	4 (5.9)	6 (2.9)	0 (0.0)	
Ocular surface parameters					
Lipid layer thickness, nm	84 (20–100)	85 (37–100)	79 (20–100)	100 (50–100)	**<0.001**
Subjective score (OSDI)	30.05 (12.50–100.00)	36.25 (14.60–66.70)	30 (12.50–100)	28 (12.50–85.40)	0.148
Schirmer's test I value, mm	9 (0–35)	7 (0–10)	9 (0–35)	9 (0–25)	0.162
TBUT, s	3 (0–15)	3 (0–4)	3 (0–15)	4 (0–15)	**0.042**
Corneal staining score (0–5)‡	1 (1–15)	1 (0–5)	1 (0–15)	2 (0–14)	0.263
Conjunctival staining score (0–10)‡	4 (1–18)	3 (1–6)	4 (0–18)	4 (0–10)	0.053
TMH, μm by FD-OCT	242 (80–484)	228 (80–345)	233 (163–484)	237 (120–415)	0.748
Presence of MGD, eyes, *n* (%)	255 (79.0)				
Obstructive type	204 (80.0)				
Hypersecretory type	51 (20.0)				
Presence of lid margin inflammation, *n* (%)	146 (57.3)		117 (55.9)	32 (62.8)	0.354†
MGD grade (0–4)	2 (1–4)		2 (1–4)	2 (1–3)	0.757
Lid margin abnormality (0–4)	2 (1–4)		2 (1–4)	1 (1–3)	**0.049**
Meibomian gland expressibility (0–3)	1 (0–3)		1 (0–3)	0 (0–0)	**<0.001**
Meibum quality (0–24)	7 (0–22)		7 (0–22)	7 (0–12)	0.489

Bold numbers indicate statistically significant results.
* Kruskal-Wallis test.
† Fisher exact tests.
‡ Oxford staining score.

median (range) LLT was 84 (20–100) nm. Among the DES patients, 255 subjects (79.0%) fulfilled the diagnostic criteria for MGD, which was classified as either obstructive (80.0%) or hypersecretory MGD (20.0%). Of the patients with DES with MGD, 146 (57.3%) had lid margin inflammation, and the median MGD grade was 2, which was based on the three lid parameters: lid margin abnormality score (median grade, 2), expressibility (median grade, 1), and meibum secretion quality (median grade, 7).

The patients with DES were classified into three subgroups: DES without MGD ($n = 68$), DES with obstructive MGD ($n = 204$), and DES with hypersecretory MGD ($n = 51$). The comparison of clinical variables between the subgroups of DES showed significant differences in the median value of the LLT ($P < 0.001$). Among the three subgroups, age, previous history of refractive surgery, cataract surgery, contact lens use, and TBUT were also significantly different ($P < 0.050$). Between the two MGD subgroups, the obstructive MGD subgroup had a higher lid margin abnormality score and less expressible meibomian glands ($P < 0.050$; Table 2).

Factors Associated With Lipid Layer Thickness in the Dry Eye Syndrome Group

In the univariate analysis, age, sex, history of refractive surgery, cataract surgery, contact lens use, and ocular surface and meibomian gland parameters were included as independent variables, and LLT was analyzed as a dependent variable. Increased age and female sex were positively associated with LLT, and a history of refractive surgery, cataract surgery, and contact lens use were negatively associated with the LLT in DES. Several ocular surface parameters and meibomian gland parameters were significantly associated with LLT.

In a multivariate analysis to determine the independence of the effects, increased age, and female sex were significantly related to increased LLT ($\beta = 0.282$, $P = 0.005$ for age and $\beta = 11.493$, $P < 0.001$ for female sex). A history of refractive surgery or cataract surgery was negatively associated with LLT ($\beta = -15.678$, $P < 0.001$ and $\beta = -8.996$, $P = 0.014$, respectively). Among the ocular surface parameters, the only factor that independently influenced LLT was Schirmer's test I value, which was negatively associated with LLT ($\beta = -0.642$, $P = 0.017$). Dry eye syndrome with hypersecretory MGD and lid margin inflammation independently associated with increased LLT ($\beta = 11.299$, $P = 0.001$ and $\beta = 12.747$, $P = 0.001$, respectively). In addition, lid margin abnormality scores negatively associated with LLT ($\beta = -5.453$, $P = 0.005$; Table 3).

In a subgroup analysis, DES with obstructive MGD ($n = 204$) was associated with the same factors that were associated with LLT, and MGD grade and meibomian gland expressibility were also negatively associated with LLT ($\beta = -6.738$, $P = 0.023$ for MGD grade and $\beta = -5.452$, $P = 0.005$ for expressibility, respectively; Table 4).

Table 3. Univariate and Multivariate Linear Regression Analysis to Evaluate the Impact of Clinical Variables Including Meibomian Gland Characteristics on Lipid Layer Thickness in Dry Eye Syndrome Group

	Univariate Model		Multivariate Model	
Variables	**Beta (SE)**	***P* Value**	**Beta (SE)**	***P* Value**
Age, y	**0.406 (0.069)**	**<0.001**	**0.282 (0.099)**	**0.005**
Sex				
Male	1 (Ref)		1 (Ref)	
Female	**14.636 (2.787)**	**<0.001**	**11.493 (2.997)**	**<0.001**
Ocular history				
No history	1 (Ref)		1 (Ref)	
Refractive surgery	**−19.118 (3.656)**	**<0.001**	**−15.678 (4.329)**	**<0.001**
Cataract surgery	**−8.118 (3.656)**	**0.027**	**−8.996 (3.652)**	**0.014**
Contact lens use	−15.567 (7.107)	0.029	−7.551 (7.858)	0.337
Ocular surface parameters				
Subjective score (OSDI)	**0.170 (0.060)**	**0.005**	0.003 (0.059)	0.954
Schirmer's test I value, mm	**−0.616 (0.175)**	**0.001**	**−0.379 (0.176)**	**0.032**
TBUT, s	0.110 (0.477)	0.818		
Corneal staining score (0–5)*	**1.373 (0.426)**	**0.001**	0.074 (0.550)	0.893
Conjunctival staining score (0–10)*	**0.966 (0.376)**	**0.011**	0.280 (0.531)	0.599
TMH, μm by FD-OCT	−0.014 (0.017)	0.407		
MGD type				
Obstructive type	1 (Ref)		1 (Ref)	
Hypersecretory type	**13.524 (3.372)**	**<0.001**	**11.299 (3.213)**	**0.001**
Presence of lid margin inflammation				
No	1 (Ref)		1 (Ref)	
Yes	**13.222 (2.511)**	**<0.001**	**12.747 (3.808)**	**0.001**
MGD grade (0–4)	**4.111 (1.204)**	**0.001**	0.494 (3.462)	0.887
Lid margin abnormality (0–4)	**3.654 (1.022)**	**<0.001**	**–5.453 (1.919)**	**0.005**
Meibomian gland expressibility (0–3)	−0.537 (1.433)	0.708		
Meibum quality (0–24)	**0.939 (0.262)**	**<0.001**	0.132 (0.645)	0.838

Bold numbers indicate statistically significant results.
* Oxford staining score.

Discussion

Obstructive MGD can be diagnosed through biomicrosopic examination with a manual test of the meibomian gland expressibility or by a meibography to visualize and assess the degree of gland dropout.[20] Because previous studies reported the correlation between a thinner LLT and fewer expressible meibomian glands, a direct quantification of LLT measurements using an interferometer has been recently introduced as a useful tool for the diagnosis of obstructive MGD.[5–9] Although previous report suggested a 75-nm cut-off value for the LLT measurement using the LipiView interferometer for the detection of obstructive MGD, the discrepancy between the LLT value and the clinical findings suggests that further validation of the clinical application of the value is required. Therefore, the main purpose of our study was to determine the effects of clinical variables on lipid layer thickness in normal subjects and in patients with DES, respectively. We believe that these factors may be potential confounding factors for the actual LLT or the LLT values reported by the LipiView.

In this study, the median value of LLT was 67 nm in the normal group and 84 nm in the DES group. Figure 2 reveals the reason for this difference by stratifying the LLT values by each subgroup and by age and sex. Normal subjects were mostly younger than dry eye patients, and dry eye patients included hypersecretory MGD patients, which explain why a higher LLT was observed in dry eye patients. These LLT values of the DES group were higher than those reported in previous studies[5,6,21,22] carried out in populations with different age distributions. Asian (particularly Korean) ethnicity and the inclusion of hypersecretory MGD in our study might have contributed to these differences. Thus, for the determination of a generalized absolute value for the diagnosis of MGD, the effects of the heterogeneity of the groups should be considered. However, in Figure 2, the median LLT values of normal subjects were higher than those of obstructive MGD patients with the same age range and sex.

In the regression analyses in our study, the LLT increased with age increase, and age was a strong influential factor in LLT in both the normal and DES groups. Except age, there were no other factors related to the LLT in normal eyes; however, in eyes with DES, significant correlations were found between the LLT and other variables. In DES, after adjusting other demographic factors and the ocular surface and meibomian gland parameters, women had a greater LLT than men. Previous studies showed that the tear film stability decreases in old age,[23,24] and Maïssa and Guillon[25] demonstrated that age and sex are significant factors that influence the characteristics of the tear film lipid layer and tear film dynamics using a Tearscope. These authors demonstrated the significantly poorer quality of the lipid layer and thinner lipid layer thickness in women who were 45 years or older. The thickness of the lipid layer was classified based on its appearance using the Tearscope in their study; however, in our study, the LipiView interferometer, which is capable of quantifying LLT, was used. Older female patients often had a poorer quality lipid layer with contamination; however, LLT measurements using the interferometer were thick. Not only the numeric values of

Table 4. Univariate and Multivariate Linear Regression Analysis to Evaluate the Impact of Clinical Variables Including Meibomian Gland Characteristics on Lipid Layer Thickness in Dry Eyes Syndrome With Obstructive MGD

	Univariate Model		Multivariate Model	
Variables	**Beta (SE)**	***P* Value**	**Beta (SE)**	***P* Value**
Age, y	**0.562 (0.107)**	**<0.001**	0.257 (0.137)	0.062
Sex				
Male	1 (Ref)		1 (Ref)	
Female	**20.810 (4.324)**	**<0.001**	**14.910 (4.466)**	**0.001**
Ocular history				
No history	1 (Ref)		1 (Ref)	
Refractive surgery	**−26.832 (5.588)**	**<0.001**	**−18.486 (6.384)**	**0.004**
Cataract surgery	**−13.925 (5.644)**	**0.014**	**−14.673 (5.475)**	**0.007**
Contact lens use	**−22.885 (10.777)**	**0.034**	−6.046 (11.260)	0.591
Ocular surface parameters				
Subjective score (OSDI)	**0.247 (0.097)**	**0.011**	−0.054 (0.094)	0.565
Schirmer's test I value, mm	**−0.996 (0.273)**	**<0.001**	**−0.642 (0.270)**	**0.017**
TBUT, s	−0.208 (0.758)	0.784		
Corneal staining score (0–5)*	**1.373 (0.426)**	**0.001**	0.074 (0.550)	0.893
Conjunctival staining score (0–10)*	**0.966 (0.376)**	**0.011**	0.280 (0.531)	0.599
TMH, μm by FD-OCT	−0.025 (0.026)	0.325		
Presence of lid margin inflammation				
No	1 (Ref)		1 (Ref)	
Yes	**20.803 (4.040)**	**<0.001**	7.514 (4.405)	0.088
MGD grade (0–4)	**−5.806 (1.616)**	**<0.001**	**−6.738 (2.962)**	**0.023**
Lid margin abnormality (0–4)	−17.274 (9.820)	0.079	−5.269 (4.229)	0.086
Meibomian gland expressibility (0–3)	**−13.664 (3.293)**	**<0.001**	**−5.453 (1.919)**	**0.005**
Meibum quality (0–24)	**0.939 (0.262)**	**<0.001**	0.372 (0.696)	0.594

Bold numbers indicate statistically significant results.
* Oxford staining score.

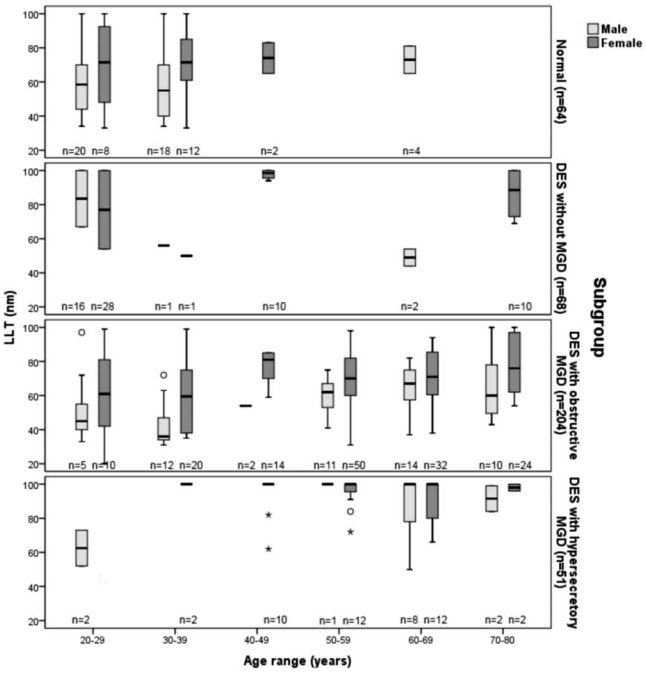

Figure 2. Box plot of LLT stratified by each of the subgroups and by age and sex. *Horizontal lines in the boxes* indicate the median (second quartile); box limits show third quartile (*top*) and first quartile (*bottom*). Outliers (1.5–3× interquartile range) are indicated as circles, and extremes (>3× interquartile range) are indicated as *asterisks*. Maximum and minimum values are indicated by the *top and bottom whisker ends*, respectively.

LLT but also the quality of the lipid layer with contamination needed to be evaluated. Therefore, the greater LLT in aged eyes and in women found in our study may not be interpreted as a positive result; these confounding factors should be accounted for when interpreting the meaning of the LLT value. The low LLT of normal and young subjects cannot be considered a negative result. Therefore, we suggest that without consideration of the affecting factors, an absolute LLT for use as a diagnostic tool cannot be determined. Additionally, an internally normalized database based on age and sex should be developed for using the LLT value as a diagnostic parameter.

Histories of refractive and cataract surgery were independently related to the decrease in LLT. Previous studies[10,26] reported that tear stability, lid margin abnormalities, and meibum expressibility worsened after cataract surgery and that the operation itself and postoperative inflammation may cause postoperative ocular discomfort by influencing meibomian gland function without the accompanying structural changes that can be seen using meibography. Chronic postrefractive surgery tear dysfunction, which includes postoperative neurotrophic disease, tear instability, true aqueous tear deficiency, and neuropathic pain states, has also been reported.[27] Several studies also reported that chronic dry eye disease after refractive surgery can be attributed to lipid layer deficiency.[28,29] In one such study, subjects who presented with persistent dry eyes for more than 1 year postoperatively showed signs of lipid layer deficiency. Their symptoms and lipid layer thickness improved on lid warming, suggesting that MGD was the underlying cause of their dry eyes.[29] These ocular surface changes after cataract and refractive surgery can be explained by the decrease in LLT based on our present results.

Arita et al.[30] investigated the influence of rigid gas-permeable lens wear with an average duration of 12.3 ± 7.2 years on the meibomian glands and reported higher meiboscores than those of controls. In our study, the significant association between contact lens use and LLT in the univariate analysis was not maintained after correcting for other factors. Because our contact lens users often used soft contact lenses for a relatively short period and they represented a small portion of all subjects, additional data is needed to determine the effect of contact lens use on the LLT.

After adjusting for other demographic factors, ocular surface, and meibomian gland parameters, a severe OSDI score was not related to a thin lipid layer in our study. However, a previous study showed that LLT has been shown to correlate better to symptoms, particularly severe symptoms.[31] Their subjects were younger than those of our study, and all of them were not dry eye patients. This study evaluated the effect of symptoms on LLT without adjusting for other variables.

An increase of the Schirmer score was significantly related to a decrease of LLT after adjusting for other factors. The correlation between tear production and meibomian gland function has been suggested in previous experimental and clinical observations,[16,32–37] but this remains controversial. Recently, a multicenter cross-sectional study showed that an increase in tear fluid production compensates for loss of the meibomian glands.[16] These results and our findings suggest that DES related to aqueous deficiency and cannot simply be distinguished from DES related to lipid deficiency, and these secretions have a complementary relationship.

Among the meibomian gland parameters, the presence of hypersecretory MGD and the presence of lid inflammation were also independently related to a thicker LLT. Additionally, larger lid margin abnormality scores were associated with a thinner LLT. A subgroup analysis of DES with obstructive MGD showed that MGD grade and expressibility were negatively associated with LLT. These findings showed that the LLT measurements using a LipiView interferometer well reflect the quantitative changes of the expressed meibum.

Additionally, we evaluated the characteristics of Koreans with DES in the study group, who visited a tertiary referral clinic during the winter (mean temperature: −1.0 ± 3.6°C; relative humidity: 52.6 ± 13.8% in Seoul). Among eyes with DES, 79.0% fulfilled the diagnostic criteria for MGD, which was classified into obstructive (80.0%) and hypersecretory MGD (20.0%). Other population-based studies have estimated the prevalence of MGD to be approximately 60% in Japanese and Chinese populations, which is higher than that of a previous western study.[4] An elderly Korean population-based study also showed an approximately 50% prevalence of MGD, and there was no significant difference between participants with and without DES.[38] However, in a recent clinic-based patient cohort,[39] 86% of DES cases showed evidence of MGD, and the evaporative form of DES was far more common than pure aqueous dry eye, this finding was similar to our results.

Our data also showed the characteristics of the hypersecretory MGD subgroup. The median age was higher, and a history of refractive surgery or contact lens use appeared to be rare in patients with this type MGD. These patients had a lower lid margin abnormality score and more expressible meibomian glands compared with patients with obstructive MGD. So far, comprehensive diagnostic criteria for this type of MGD have not yet been established and in many previous reports on MGD, individuals whose eyes showed excessive meibomian lipid secretion were also excluded. High-delivery states (i.e., seborrheic MGD) are currently diagnosed only by an excessive expression of meibum and slit-lamp examination of the lid margins,[17,18] similar to our definition. As certain cases of obstructed glands with inspissated secretions may suddenly open on manual expression with a release of a large volume of meibum, more definite criteria of hypersecretory MGD are needed, and the lack of comprehensive criteria was likely a limitation of our study.

In conclusion, automated assessment of the lipid layer thickness using a novel interferometer is significantly affected by demographic factors such as age, sex, ocular surgical history, and the MGD type. Therefore, to use this value as a diagnostic tool for evaluating ocular surface diseases including DES and MGD, we should consider all of the affecting factors. There is a need to establish an internally normalized database based on the affecting factors, rather than using a single absolute LLT value.

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