Evaluating Dry Eye Disease Subtypes using Tear Interferometry

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Evaluating Dry Eye Disease Subtypes using Tear Interferometry

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**Department of Medicine
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ABSTRACT

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Purpose: This study evaluates the diagnostic significance of inferior (LLT_{inf}) and superior (LLT_{sup}) corneal lipid layer thickness (LLT) in differentiating meibomian gland dysfunction (MGD), aqueous deficiency (AD), and fluorescein tear break-up patterns (FTBUPs).

Methods: A cross-sectional study of 310 eyes in 310 dry eye disease (DED) patients was conducted. LLT_{inf} was measured via tear interferometer, and LLT_{sup} via LED plate. Conventional DED parameters, including Ocular Surface Disease Index questionnaire, fluorescein tear break-up time, corneal staining score, and tear meniscus height, meibomian gland functionality, and FTBUPs were analyzed.

Results: Within low LLT_{inf}, decreased meibomian gland functionality was observed. LLT_{inf} correlated negatively with tear meniscus height, and $LLT_{inf} \geq 90$ nm was associated with a higher proportion of AD, particularly when LLT_{sup} was low. However, the spot break pattern of FTBUPs influenced LLT measurement as a confounding factor when LLT_{sup} was high. The combined LLT_{inf} and LLT_{sup} assessment improved AD prediction (AUC = 0.77) compared to LLT_{inf} alone (AUC = 0.56 and 0.58, respectively), though it only partially enhances some MGD grade prediction.

Conclusions: Tear interferometry reflects not only lipid secretion but also muco-aqueous conditions and their distributions. Combined inferior and superior corneal LLT assessment enhances DED subtype classification and provides a more comprehensive evaluation of tear film.

Key Words: aqueous deficiency, dry eye disease, lipid layer thickness, meibomian gland dysfunction, tear film-oriented diagnosis, tear interferometry

1. INTRODUCTION

1.1. Research Background

1.1.1. Dry eye disease

Dry eye disease (DED) is a spectrum of disorders caused by various etiologies, including ocular and systemic conditions such as allergic march, Sjögren syndrome, Stevens-Johnson syndrome, and graft-versus-host-disease, as well as age, hormonal change, lifestyle, and environmental factors that contribute to either a transient or chronic phase of both quantitative and qualitative tear film dysfunction.[1] Tear film dysfunction leads to ocular surface tissue damage due to reduced lubrication and increased friction, triggering the activation of inflammatory signaling pathways. This inflammation, in turn, disrupts tear stability, further exacerbating DED and perpetuating its vicious cycle.[2]

1.1.2. Classification of dry eye disease

In the 2017 Dry Eye Workshop II (DEWS II), DED was categorized into two primary types: aqueous-deficient and evaporative.[1] This classification was based on the concept of the tear components, specifically the aqueous and lipid layers. The Asian Dry Eye Society (ADES) further refined this classification by incorporating the mucin dysfunction, expending the categorization into three primary types.[3]

1.1.3. Component-based assessments in dry eye disease

The component-based perspective has highlighted the importance of assessing individual tear components in addition to general DED evaluations, such as tear break-up time, tear osmolarity, and ocular surface staining scores. In addition to conventional “static” assessments, there have been efforts to evaluate the “dynamic” aspects of the tear film, including the blinking exercise and the course of tear film distribution. Representative methods include fluorescein tear break-up pattern (FTBUP) analysis and tear interferometric lipid layer assessment.[4,5] FTBUP characterizes break-up patterns within the aqueous layer, revealing distinct patterns associated with specific tear component abnormalities. Tear interferometry evaluates the lipid layer

thickness (LLT) of the tear film through the interference colors.

1.1.4. Tear interferometry and lipid layer thickness

A normal LLT typically ranges from 40-50 to 90-100 nm.[6] In cases of low-delivery meibomian gland dysfunction (MGD), where there is decreased secretion of meibum, the primary component of the tear film lipid layer (TFLL), LLT falls below 40–50 nm. However, LLT measurement is not solely determined by the lipid volume but is also influenced by blinking dynamics and the distribution of the aqueous fluid.[7] During eyelid closure, the lipid layer is pushed toward the lower eyelid margin, while contraction of the orbicularis oculi muscle compresses the meibomian glands, releasing meibum. Upon eye opening, the secreted meibum spreads upward, following the movement of the aqueous fluid.

1.1.5. The role of tear components in tear interferometry

In a healthy tear film, this distribution occurs rapidly and uniformly across the ocular surface, leading to an even lipid layer.[7] However, in the presence of aqueous deficiency, the upward movement of aqueous fluid is restricted, impairing lipid spreading and resulting in an uneven lipid layer.[8] This aqueous deficiency leads to lipid accumulation above the lower eyelid margin, causing locally elevated LLT, which is observed in the inferior cornea through tear interferometry.[9] Notably, a high LLT in the inferior cornea does not necessarily indicate seborrheic conditions or increased LLT across the entire ocular surface, emphasizing the importance of measurement location and distribution when assessing LLT.

1.2. Limitation of Previous Studies

In most recent clinical studies on LLT, LLT has been analyzed using commercially available tear interferometers. However, depending on the interferometry platform, LLT has typically been provided as a single metric in a limited area, either in the lower cornea or at the central cornea.[10-12] As a result, comprehensive evaluation of the entire cornea, from superior to inferior regions, has been lacking, and the analysis of LLT distribution, an aspect that could offer more critical insights into tear film dynamics, has been largely excluded. These limitations have led to

differing results in studies with small sample sizes and wide statistical variation, ultimately contributing to the perception that tear interferometry has limited applicability in individualized patient assessments.

1.3. Purpose of Study

This study aims to introduce a novel diagnostic concept of tear dynamics using tear interferometry in inferior and superior cornea and investigate its role in the detailed classification of DED. Building on current assessments of component-based DED, this study aims to conduct a detailed analysis of the tear interferometric lipid layer in both the superior and inferior cornea.

2. MATERIALS & MATHODS

2.1. Study Design

This cross-sectional study conducted at Severance Hospital, Yonsei University College of Medicine, and enrolled consecutive DED patients from January 2021 to December 2024. The study protocol was approved by the Institutional Review Board of Severance Hospital (IRB approval number: 2024-3494-001) and adhered to the tenets of the Declaration of Helsinki.

2.2. Subjects

The study subjects were DED patients with following criteria. The inclusion criteria were (1) age ≥20, (2) Ocular Surface Disease Index (OSDI) ≥13 scores (mild and/or over), and (3) fluorescence tear break-up time (TBUT) ≤10 seconds. The exclusion criteria were history of ocular trauma or surgery within 6 months, history of contact lens within 3 months, and history of dry eye treatments except preservative-free artificial tear within 3 months.

2.3. Sample Size

The sample size calculation was based on a previous study that analyzed dry eye subtypes and LLT of the inferior cornea.[13] The mean LLT in the inferior cornea was reported as follows: (1) aqueous-deficient subtype: 97 ± 6 nm (n = 11), (2) evaporative subtype: 72 ± 25 nm (n = 142), (3) mixed subtype: 71 ± 27 nm (n = 144), and (4) unclassified dry eye: 74 ± 11 nm (n = 12). These values were used as the basis for sample size estimation. The effect size (Cohen's f) was calculated as 0.19, using the weighted mean and weighted variance based on the number of subjects in each group. Since normality assumptions might be violated, the effect size was adjusted using the Rank-Biserial Correlation from the Kruskal-Wallis test. To approximate an equivalent effect size, Cohen's f was converted by dividing by 0.8, yielding an adjusted f of 0.24. With a significance level (α) of 0.05 and power (1−β) of 0.95, the estimated required sample size was 310 subjects.

2.4. Tear Interferometry and Lipid Layer Thickness

Tear interferometry was performed using two methods: a non-invasive, commercially available interferometer (TearScience™ LipiView® II Ocular Surface Interferometer, Johnson & Johnson Vision, Jacksonville, Florida, United States) for the inferior cornea and a manual LED plate for the superior cornea. Both techniques utilize specular reflection of white light from the TFLL to assess its color and uniformity.

2.4.1. Tear interferometer

LLT obtained from the tear interferometer represents the LLT in the inferior cornea and the average LLT is defined as inferior corneal LLT (LLT_{inf}) (**APPENDICES 1**). During the 20-second video recording, LLT_{inf} was measured repeatedly. To ensure consistency, the average values were used for individual measurements. When the number of measurements exceeded two, both upper and lower extreme values were excluded from the calculations. Patients with highly discontinuous or irregular LLT patterns due to poor cooperation, as well as those with a C-factor below 0.90, an indicator of measurement reliability in the graphical summary, were excluded. The detailed methodology followed that of a previously published study.[9] LLT_{inf} was classified into three grades: (1) grade L ($LLT_{inf} < 40$ nm), (2) grade M ($40 \text{ nm} \leq LLT_{inf} < 90$ nm), and (3) grade H ($90 \text{ nm} \leq LLT_{inf}$).[5,6]

2.4.2. Manual lipid layer thickness measurement

For measuring superior corneal LLT (LLT_{sup}), a whitish LED plate was used under slit-lamp examination, followed by anterior segment photography.[14] The superior cornea was illuminated with the LED plate and classified into three grades using a modified version of a previously established classification system (**APPENDICES 2**):[15] (1) grade L (dark, uniform distribution), (2) grade M (gray, uniform or nonuniform distribution), and (3) grade H (colored, nonuniform distribution).

The intra-/interclass correlation coefficient (ICC) of LLT_{sup} among three independent examiners using anterior segment photography was 0.791 [95% confidence interval (CI) 0.642-0.941] (**APPENDICES 3**).

2.4.3. Lipid layer thickness group

The LLT group was categorized into nine (3 × 3) subgroups based on the LLT grades of LLT_{inf} and LLT_{sup}. Each group was labeled using the format Group $_{inf\text{-}sup}$, where "inf" represents the LLT_{inf} grade and "sup" represents the LLT_{sup} grade. For example, if LLT_{inf} was grade H and LLT_{sup} was grade M, the group was labeled as Group $_{H\text{-}M}$.

2.5. Dry Eye Disease Assessments

2.5.1. Conventional dry eye assessments

Conventional DED assessments included OSDI, TBUT, non-invasive keratographic tear break-up time (NIKBUT) measured using the Keratograph® 5M (Oculus, Wetzlar, Germany), and corneal staining score (CSS) based on the ocular surface staining score of the Sjögren's International Collaborative Clinical Alliance (SICCA).[16-18]

2.5.2. Aqueous volume assessments

Tear volume was measured with the Schirmer I test without anesthesia and tear meniscus height (TMH) at the central tear meniscus area using Keratograph® 5M.[19] Aqueous deficiency was defined as Schirmer I test ≤ 5 mm or TMH <200 μm.[1]

2.5.3. Meibomian gland assessments

Meibomian gland functionality was evaluated in the central eight glands of the upper and lower eyelids following the guidelines of the 2011 International Workshop on MGD.[20] Meibomian gland expression (MGE) was measured at a pressure of 0.3 pounds per square inch (psi; approximately 15 mmHg) using a commercially available instrument, the Meibomian Gland Evaluator (Johnson & Johnson Vision, Jacksonville, Florida, United States; **APPENDICES 4**), to ensure standardized and equivalent pressure application.[21] MGE was categorized into four grades: (1) grade 0 (all glands expressible, none), (2) grade 1 (50% and over of glands expressible, minimal to mild), (3) grade 2 (less than 50% of glands expressible, moderate), and (4) grade 3 (no glands expressible, severe). Meibum quality (MQ) was assessed as the average value of meibum feature scores: (1) grade 0 (clear fluid), (2) grade 1 (cloudy fluid), (3) grade 2 (cloudy particulate

fluid), and (4) grade 3 (toothpaste-like secretion or no secretion). The following features were assessed for lid margin abnormalities: (1) meibomian gland plugging, (2) telangiectasia, (3) anterior shift of mucocutaneous junction (MCJ), (4) notching, and (5) lid margin desquamation. MGD grade was assigned according to the most severe finding among MGE, MQ, and lid margin abnormalities.[22]

2.5.4. Fluorescein tear break-up patterns

FTBUPs were evaluated using slit-lamp examination videoclips.[4] Three independent examiners assessed the videoclip, and the ICC was 0.772 [95% CI 0.666–0.876] (**APPENDICES 2**).

2.6. Statistical Analysis

For the comparison of LLT groups, nonparametric methods were chosen due to the violation of normality and homogeneity of variance assumptions. The Kruskal-Wallis test was used for continuous variables, the Jonckheere-Terpstra test was applied for ranked variables, and the chi-square test was used for nominal variables. Dunn's test was conducted as a post-hoc analysis following the Kruskal-Wallis test. For the correlation analysis of variables, Spearman's rho correlation test was conducted.

To analyze the relative availability of concomitant measurement of inferior and superior corneal LLT, Receiver Operating Characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) was calculated. A random forest classifier was used to analyze the models. The predictive performance of LLT_{inf} alone was compared to a model incorporating both LLT_{inf} and LLT_{sup} in (1) distinguishing MGE grade 0 from grades 1, 2, and 3, (2) distinguishing MGE grades 0 and 1 from grades 2 and 3, and (3) predicting aqueous deficiency.

A significant level of p-value < 0.05 was considered statistically significant, and all statistical tests were performed at a 95% confidence level.

3. RESULTS

3.1. Characteristics of Subjects

A total of 310 eyes from 310 patients were included in this study. No subjects were assigned to Group $_{L\text{-}H\ (inf\text{-}sup)}$, and analyses were conducted on the other eight groups. The distribution of subjects across these groups is presented in **Table 1**.

Table 1. Names and number of lipid layer thickness (LLT) groups

	LLT group	LLT_{sup}		
		Grade L (Dark, uniform)	Grade M (Gray, uniform and non-uniform)	Grade H (Colored, non-uniform)
LLT_{inf}	Grade L (LLT < 40 nm)	Group $_{L\text{-}L}$ N = 42	Group $_{L\text{-}M}$ N = 28	Group $_{L\text{-}H}$ N = 0
	Grade M (40 nm ≤ LLT < 90 nm)	Group $_{M\text{-}L}$ N = 44	Group $_{M\text{-}M}$ N = 43	Group $_{M\text{-}H}$ N = 32
	Grade H (LLT ≥ 90nm)	Group $_{H\text{-}L}$ N = 49	Group $_{H\text{-}M}$ N = 34	Group $_{H\text{-}H}$ N = 38

The mean age of study subjects was 50.0 ± 15.0 years, and 71.0 % were female. The mean OSDI score was 28.1 ± 12.6, TBUT was 2.6 ± 1.9 seconds, and CSS was 0.5 ± 1.1 as detailed in **APPENDICES 5**.

3.2. Inferior Corneal Lipid Layer Thickness and Dry Eye Assessments

3.2.1. Conventional dry eye assessments

OSDI did not show a significantly difference among LLT_{inf} grades (p = 0.318). TBUT were

longest in LLT_{inf} grade M (2.6 ± 1.8 seconds), compared to grade L (1.9 ± 1.5 seconds, $p = 0.013$) and grade H (1.9 ± 1.6 seconds, $p < 0.001$), but did not differ significantly between grade L and grade H ($p = 0.348$). CSS was highest in LLT_{inf} grade H (0.8 ± 1.2), compared to grade L (0.3 ± 1.1, $p = 0.002$) and grade M (0.4 ± 1.0, $p = 0.013$), but there was no significant difference between grade L and grade M ($p = 0.455$) (**Figure 1**).

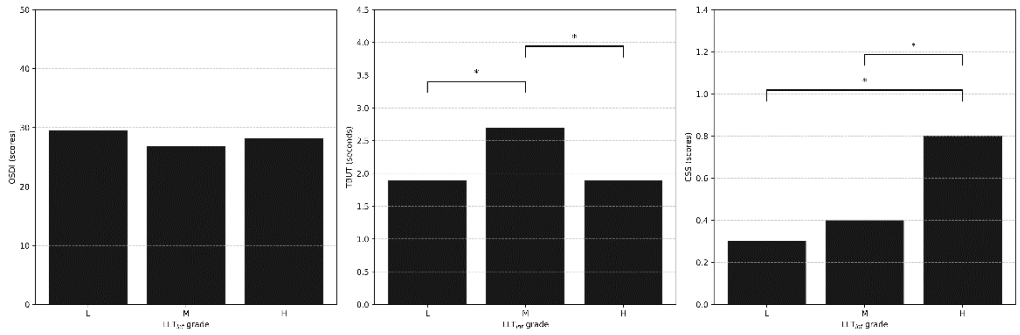

Figure 1. Ocular Surface Disease Index (OSDI, left) score, fluorescein tear break-up time (TBUT, middle), and corneal staining score (CSS, right) of inferior corneal lipid layer thickness (LLT_{inf}) grades. The bar charts describe the mean value of OSDI, TBUT, and CSS among LLT_{inf} grades. There was no difference in OSDI among LLT_{inf} grades. TBUT was longer in LLT_{inf} grade M compared to grade L and grade H. CSS was worse in LLT_{inf} grade H compared to grade L and grade M. * p-value < 0.05.

3.2.2. Meibomian gland functionality

MGE was lowest in LLT_{inf} grade L (2.2 ± 0.6), showing worse function than in grade M (1.3 ± 0.9, $p < 0.001$) and grade H (1.1 ± 0.9, $p < 0.001$). The correlation coefficient between LLT_{inf} and MGE was -0.254 (p <0.001). MQ was also worst in LLT_{inf} grade 0 (2.6 ± 0.8), compared to grade M (2.1 ± 0.9, $p = 0.003$) and grade H (2.0 ± 0.8, $p < 0.001$). The correlation coefficient between LLT_{inf} and MQ was -0.150 (p <0.001) (**Figure 2A**).

3.2.3. Aqueous volume

TMH was highest in LLT_{inf} grade L (228.7 ± 48.0 μm), followed by grade M (208.9 ± 46.7 μm, $p = 0.023$) and grade H (202.3 ± 433.2 μm, $p = 0.002$). Schirmer I test values were 7.5 ± 6.0 mm in LLT_{inf} grade L, compared to grade M (5.6 ± 4.0 mm, $p = 0.016$) and grade H (5.3 ± 3.6 mm, $p = 0.004$). The correlation coefficients of TMH and Schirmer I test with LLT_{inf} were -0.137 ($p <$

0.001) and -0.089 (p = 0.028), respectively (**Figure 2B**).

3.2.4. Fluorescein tear break-up patterns

FTBUPs differed significantly among LLT_{inf} grades ($p < 0.001$). In LLT_{inf} grade L, RB was the most predominant pattern (75.2 %), followed by SB (18.1 %). In LLT_{inf} grade M, RB was observed in 39.7 %, followed by SB (23.2 %), LB (20.0 %), and DB (16.5 %). In LLT_{inf} grade H, SB was the most predominant pattern (36.5 %), followed by LB (28.1 %) and RB (20.0 %), and SB showed diffuse distribution from LLT_{inf} group L to LLT_{inf} group H (**Figure 2C**).

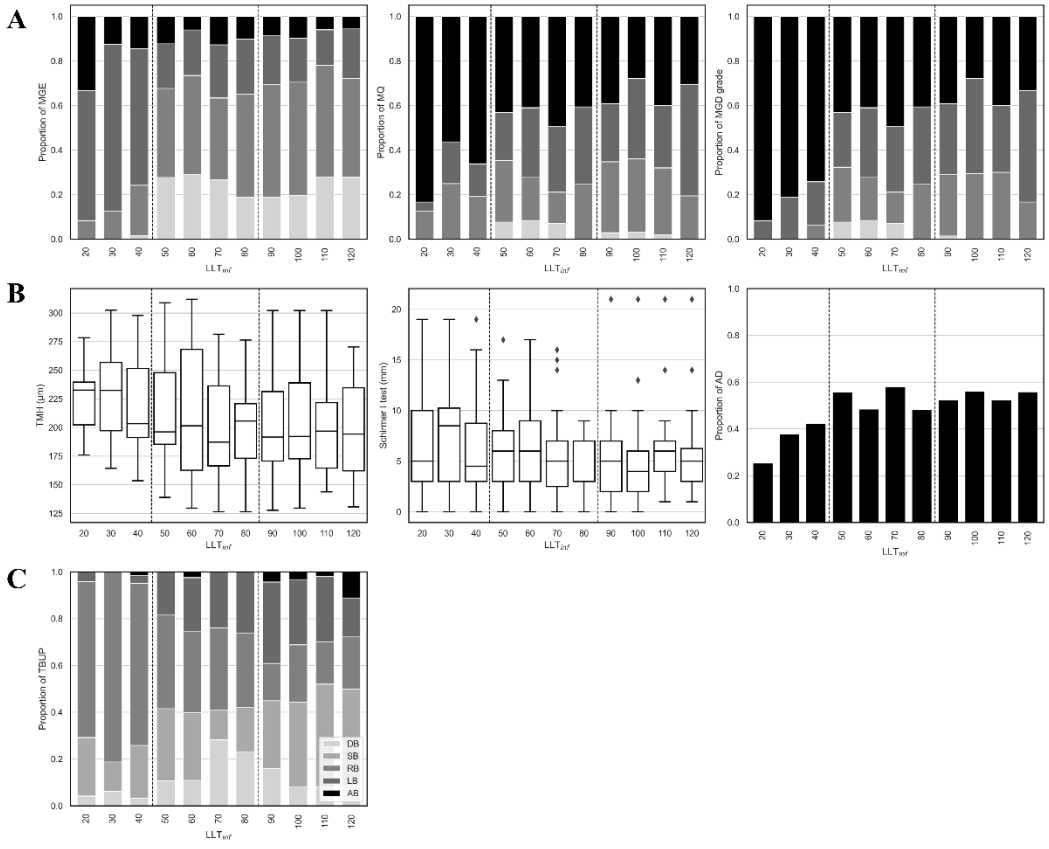

Figure 2. Meibomian gland functionality (A), aqueous volume (B), and fluorescein tear break-up patterns (FTBUP, C) of inferior corneal lipid layer thickness (LLT_{inf}) grades. The vertical dashed lines indicate the boundaries separating LLT_{inf} grades L, M, **and** H. (A) Meibomian gland functionality is represented as bar charts displaying proportions, with color gradients from light to dark indicating worsening severity from Grade 0 to Grade 3. Meibomian gland expressibility (MGE,

left), meibum quality (MQ, middle), and meibomian gland dysfunction (MGD) grade (right) were worse in LLT_{inf} grade L (LLT_{inf} under 40). (B) Tear meniscus height (TMH, left) and Schirmer I test (middle) values were highest in LLT_{inf} grade L and lowest in LLT_{inf} grade H (LLT_{inf} 90 and over). The proportion of aqueous deficiency (AD, right) was 30.0% in LLT_{inf} grade L, which was lower than LLT_{inf} grade M (LLT_{inf} between 40 and 90) and grade H. (C) FTBUPs differed significantly among LLT_{inf} grades.

3.3. Subgroup Analysis of Superior Corneal Lipid Layer Thickness

3.3.1. Conventional dry eye assessments

In LLT_{inf} grade M, OSDI was lowest in Group $_{M\text{-}M\ (inf\text{-}sup)}$ (24.0 ± 10.5) compared to Group $_{M\text{-}L}$ (31.3 ± 12.6, $p < 0.001$) and Group $_{M\text{-}H}$ (27.3 ± 10.0, $p = 0.049$). CSS was lower in Group $_{M\text{-}M}$ (0.2 ± 0.6) compared to Group $_{M\text{-}L}$ (0.6 ± 1.0, $p = 0.013$) and Group $_{M\text{-}H}$ (0.4 ± 0.6, $p = 0.158$), but the difference was not statistically significant between Group $_{M\text{-}M}$ and Group $_{M\text{-}H}$. In LLT_{inf} grade H, OSDI in Group $_{H\text{-}L}$ (32.2 ± 9.8) was worse than Group $_{H\text{-}H}$ (26.3 ± 11.5, $p = 0.018$). TBUT was shortest in Group $_{H\text{-}H}$ (1.2 ± 1.4 seconds) compared to Group $_{H\text{-}L}$ (2.2 ± 1.6 seconds, $p < 0.001$) and Group $_{H\text{-}M}$ (1.9 ± 1.4 seconds, $p = 0.005$). CSS was highest in Group $_{H\text{-}L}$ (1.0 ± 1.3) compared to Group $_{H\text{-}M}$ (0.6 ± 0.6, $p = 0.019$) and Group $_{H\text{-}H}$ (0.4 ± 0.4, $p < 0.001$) (**Figure 3**).

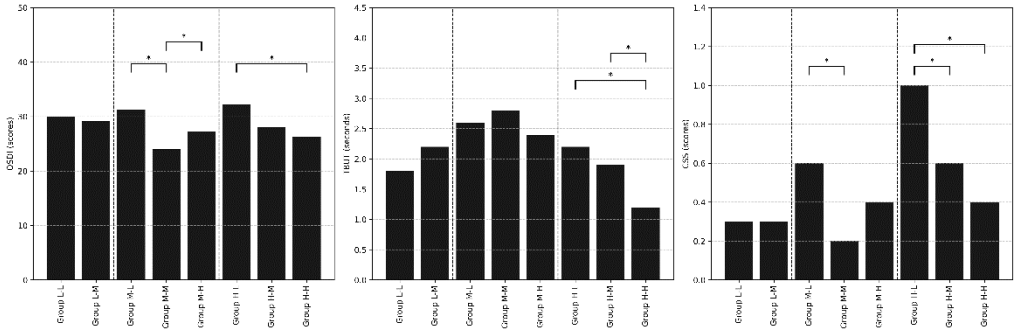

Figure 3. Ocular Surface Disease Index (OSDI, left) score, fluorescein tear break-up time (TBUT, middle), and corneal staining score (CSS, right) of superior corneal lipid layer thickness (LLT_{sup}) subgroups. OSDI, TBUT, and CSS were compared among LLT_{sup} within each inferior corneal lipid layer thickness (LLT_{inf}) grade. The vertical dashed lines indicate the boundaries separating LLT_{inf} grades L, M, and H. The group names were formatted as Group LLT_{inf} grade-LLT_{sup}. In LLT_{inf} grade L, there were no differences among subgroups in OSDI, TBUT, and CSS. In LLT_{inf} grade M, OSDI was lower in Group $_{M\text{-}M}$ compared to Group $_{M\text{-}L}$ and Group $_{M\text{-}H}$, and CSS in Group $_{M\text{-}M}$ was lower than in Group $_{1\text{-}0}$. In LLT_{inf} grade H, OSDI and CSS were worse in Group $_{H\text{-}L}$ compared to Group $_{H\text{-}M}$ and Group $_{H\text{-}H}$. Regarding TBUT, Group $_{H\text{-}H}$ had the

shortest TBUT, which was lower than in Group $_{H\text{-}L}$ and Group $_{H\text{-}M}$. * p-value < 0.05.

3.3.2. Meibomian gland functionality

MGE decreased as LLT$_{sup}$ grade decreased in LLT$_{inf}$ grade M and grade H ($p < 0.001$ and 0.016, respectively), but no significant difference was observed in LLT$_{inf}$ grade L ($p = 0.562$). MQ worsened as LLT$_{sup}$ decreased in LLT$_{inf}$ grade M ($p < 0.001$), but no significant differences were observed in LLT$_{inf}$ grades L and grade H ($p = 0.751$ and 0.410, respectively) (**Figure 4A**).

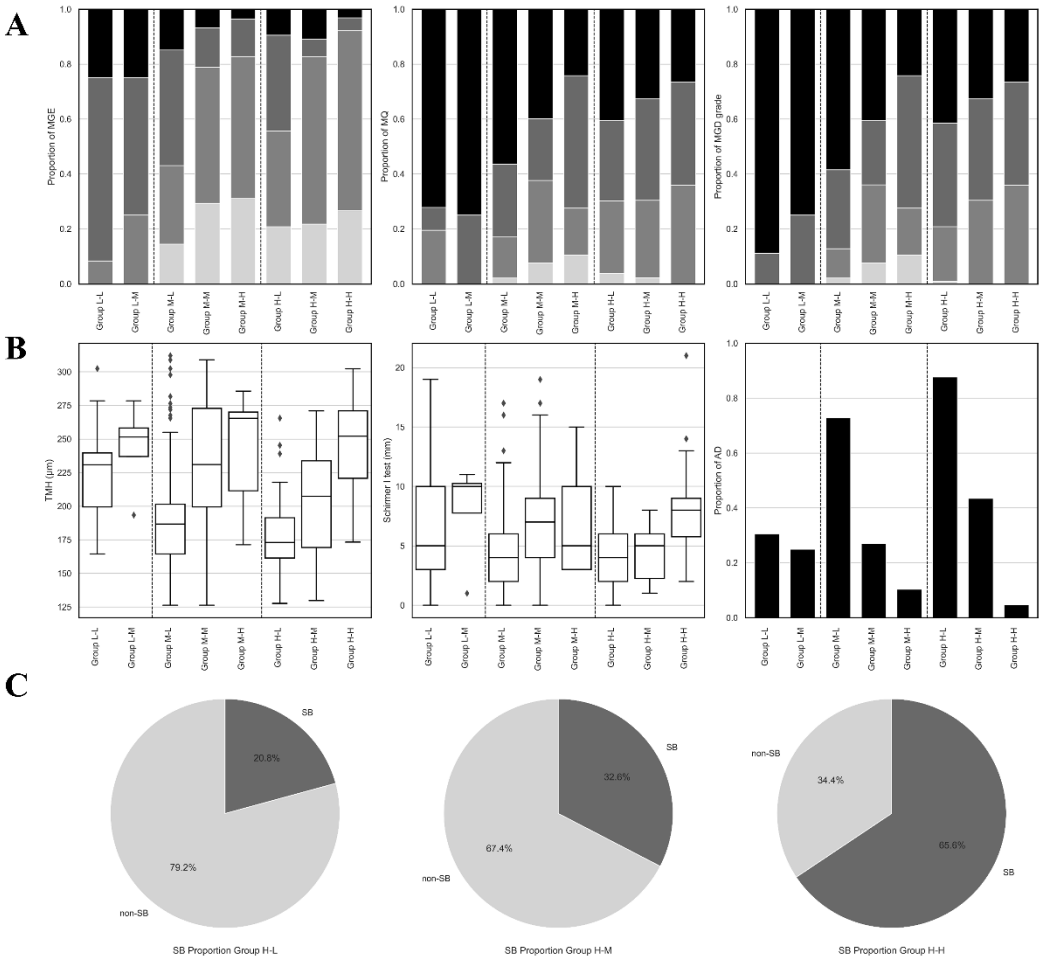

Figure 4. Meibomian gland functionality (A), aqueous volume (B) of superior corneal lipid layer thickness (LLT$_{sup}$)

subgroups and spot break (SB) proportion in LLT_{sup} subgroups of LLT_{inf} grade H. Meibomian gland functionality and tear volume were compared among LLT_{sup} within each inferior corneal lipid layer thickness (LLT_{inf}) grade. The group names were formatted as Group LLT_{inf} grade-LLT_{sup}. The vertical dashed lines indicate the boundaries separating LLT_{inf} grades L, M, and H. (A) Meibomian gland expressibility (MGE, left) decreased as LLT_{sup} grade decreased in LLT_{inf} grade M and grade H, but no difference was observed in LLT_{inf} grade L. Meibum quality (MQ, middle) worsened as LLT_{sup} decreased in LLT_{inf} grade M. (B) Both tear meniscus height (TMH, left) and Schirmer I test (middle) values increased as LLT_{sup} increased. (C) Among $LLT_{\mathbf{sup}}$ subgroups of $LLT_{\mathbf{inf}}$ grade H, the highest proportion of spot break (SB, middle) was observed in Group $_{H\text{-}H}$ (65.6%).

3.3.3. Aqueous volume

Both TMH and Schirmer I test values increased as LLT_{sup} increased in all LLT_{inf} grades (all $p < 0.001$) (**Figure 4B**).

3.3.4. Fluorescein tear break-up patterns

In LLT_{inf} grade H, the highest proportion of SB was observed in Group $_{H\text{-}H}$ (65.6%) compared to Group $_{H\text{-}L}$ and Group $_{H\text{-}M}$ ($p < 0.001$) (**Figure 4C**).

3.4. Performance of Combined LLT_{inf} and LLT_{sup} Assessment

The performances of combined LLT_{inf} and LLT_{sup} assessment in MGE, aqueous deficiency, and DWDE were compared to the performances of LLT_{inf} assessment by calculating AUC (**Figure 5**). Both in previous studies and in the present study, MGE showed a higher correlation with LLT_{inf} compared to MQ.[13] Moreover, since MGE and MQ exhibited high collinearity, MGE was selected to evaluate the performance in meibomian gland functionality.

3.4.1. Meibomian gland expressibility

The AUC for distinguishing MGE grade 0 from grade 1, 2, and 3 was 0.65 in the LLT_{inf} assessment, with no additive effect observed when incorporating LLT_{sup} measurement (AUC = 0.60) (**Figure 5, left**). However, for distinguishing MGE grade 0 and 1 from grade 2 and 3, the combined LLT_{inf} and LLT_{sup} assessment demonstrated a minimally higher AUC of 0.72 compared to LLT_{inf} alone (AUC = 0.68) (**Figure 5, middle**).

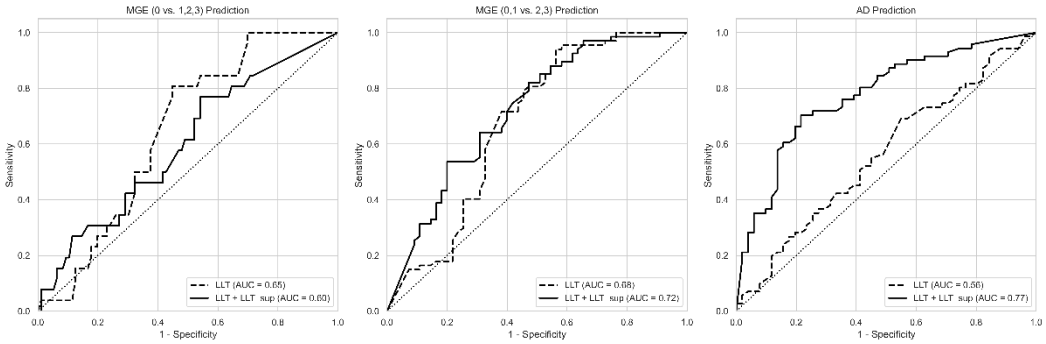

Figure 5. Comparisons of the performance between inferior corneal lipid layer thickness (LLT_{inf}) alone and the combined LLT_{inf} and superior corneal lipid layer thickness (LLT_{sup}) assessment in predicting meibomian gland expressibility (MGE, left and middle) and aqueous deficiency (AD, right). Prediction of MGE was evaluated using two classification approaches: distinguishing MGE grade 0 from grades 1, 2, and 3, and distinguishing MGE grades 0 to 1 from grades 2 to 3. The combined LLT_{inf} and LLT_{sup} assessment demonstrated higher predictive performance for AD compared to LLT_{inf} alone.

3.4.2. Aqueous deficiency

The combined LLT_{inf} and LLT_{sup} assessment showed an AUC of 0.77, demonstrating better performance compared to LLT_{inf} alone (AUC = 0.56) in predicting aqueous deficiency. The optimal thresholds for LLT_{inf} and LLT_{sup} were 78.3 and 0.05, respectively (**Figure 5, right**).

4. DISCUSSION

This study investigated the diagnostic significance of LLT measurements in both the inferior and superior cornea for evaluating DED subtype based on TFLL dynamics. The findings indicate that LLT is associated not only with lipid secretion but also with aqueous volume and its distribution. A decreased inferior corneal LLT suggests reduced lipid secretion, whereas an increased inferior corneal LLT does not necessarily indicate a seborrheic condition. To differentiate between these conditions, assessing superior corneal LLT is important. A low superior corneal LLT supports impaired lipid distribution, such as in aqueous deficiency, whereas a high superior corneal LLT suggests a seborrheic condition and may also be confounded by SB of FTBUPs (**Figure 6**).

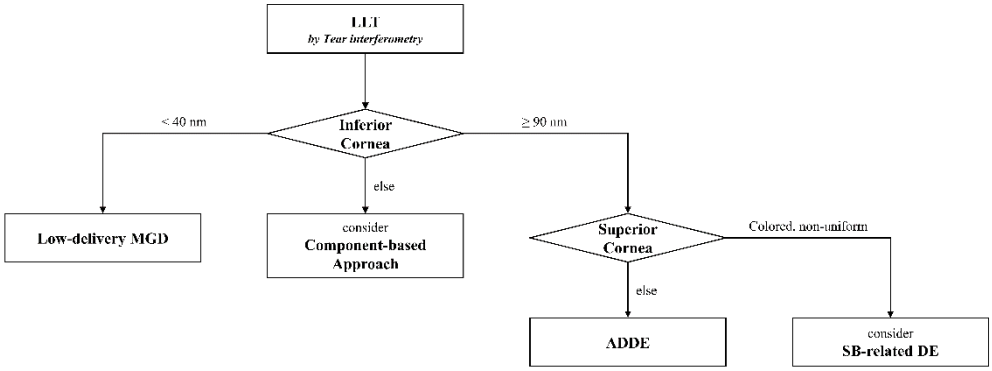

Figure 6. Clinical interpretation flowchart based on tear interferometric lipid layer thickness (LLT) measurements. When the LLT of the inferior cornea is <40 nm, low-delivery meibomian gland dysfunction (MGD) is primarily suspected. When the LLT is between 40 and 90 nm, a component-based approach is recommended to identify the underlying pathology. For LLT ≥90 nm, evaluation of the LLT pattern in the superior cornea is essential: if the superior LLT is colored and non-uniform, SB-related dry eye (DE) is suggested; otherwise, aqueous-deficient dry eye (ADDE) predominance is considered likely.

In previous studies examining LLT and DED, some results were consistent, while others varied across studies. A common finding is that DED indicators tend to be most favorable within the normal LLT range of 40–90 nm.[13,22,23] The observed trend is presumably attributable to the fact that patients exhibiting relatively normal tear film function predominantly fall within this range.

There is an issue with the relationship between LLT and meibum secretion. In most studies,

decreased meibomian gland functionality was observed in low LLT group. However, no significant difference in meibomian gland functionality was found between normal and high LLT groups,5,23 while one study reported the decreased meibomian gland functionality in high LLT group.13 These findings suggested that high LLT can be influenced by non-meibum secretion factors and may be explained by two possible mechanisms. First, decreased aqueous volume leads to impaired lipid distribution, resulting in the accumulation of lipid component in inferior corneal area and measured LLT is increased.8,9 Second, lipid compensation may occur in response to aqueous deficiency. However, this hypothesis has been challenged in a previous study, in contrast to aqueous compensation in MGD.[23]

Another issue is the relationship between LLT and tear volume. Most studies report that higher LLT is associated with lower tear volume.[5,8,9,13,15,23] Two main hypotheses have been proposed to explain this observation. One hypothesis suggests that aqueous compensation occurs in low-delivery MGD with lower LLT.[24] The other hypothesis is that LLT increases in cases of aqueous deficiency.[8,9] This study supports both hypotheses. In low LLT_{inf} groups, the proportion of aqueous deficiency was 30%, which is lower than the generally reported around 50% when accounting for pure aqueous-deficient and mixed DED subtypes.[13] Conversely, in high LLT_{inf} group, 53.7% of subjects had aqueous deficiency, which increased to 87.7% when LLT_{sup} was low. However, one study did not find a significant reduction in tear volume in groups with high LLT. [21] The study reported higher ocular surface staining scores and lower TBUT in high LLT group, and ocular surface staining scores were negatively correlated with Schirmer I test results (r = -0.302), while TBUT was positively correlated (r = 0.473). These findings suggest that the factors other than aqueous deficiency may increase LLT.

This study suggests that SB may be a confounding factor affecting study results in high LLT_{inf} group (see also **APPENDICES 6**). The previous study on FTBUPs showed that SB pattern had a shorter TBUT (1.8 ± 2.3 seconds) than other FTBUPs, except AB (0.2 ± 0.8 seconds). However, CSS of SB (1.6 ± 2.8) using the NEI scoring system was lower than that of AB (11.6 ± 3.0) and LB (5.7 ± 3.1), with no significantly difference compared to other FTBUPs.[25] Similarly, this study demonstrated a mismatch between TBUT and CSS in high LLT_{inf} group, particularly in cases with high LLT_{sup}, where the proportion of SB was higher compared to low LLT_{sup}. Nevertheless, since SB was distributed across all LLT_{inf} groups, it does not appear to be a direct inducer of a hypersecretory meibum state. Previous reports suggested that short TBUT DED, in which SB is also

considered a subtype, may be related to various factors, including mucin abnormalities, meibum change, and acute inflammation, reflecting the complex etiology of DED.[4,25] This finding suggests that one or more of these factors acted as a confounding variable in LLT measurements. Further research is needed to validate this finding.

The performance of LLT-based DED subtype prediction, with AUC values ranging from 0.6 to 0.7, cannot be considered highly accurate as a standalone diagnostic method. However, its clinical applicability may be expected in two aspects. First, by assessing the TFLL distribution across the ocular surface, it offers a new perspective for evaluating ocular surface status with other dynamic or static DED assessments. Second, it may provide a potential indirect indicator of tear film component abnormalities or possibly ocular surface abnormalities such as inflammation, which have not yet been widely integrated into clinical practice.

This study has several limitations. First, due to non-standardized distribution of variables across groups, nonparametric statistical tests were used, which limits the ability to interpret precise numerical differences between groups. However, this study did not aim to derive regression formulas or quantitative assessments but rather to establish a framework for evaluating tear film abnormalities. Second, this study did not conduct a quantitative measurement of superior corneal LLT. While manual LLT measurement of the superior cornea demonstrated acceptable reliability with an ICC near 0.8, the lack of a quantitative measurement method may have limited to provide numerical objectivity of the results.

5. CONCLUSION

Although LLT primarily reflects TFLL, it is also influenced by the muco-aqueous layers of the tear film and other potential factors. A comprehensive evaluation of both the superior and inferior cornea is important for accurately interpreting LLT findings. Understanding not only lipid secretion but also lipid distribution dynamics can provide valuable insights into tear film physiology and contribute to a more refined evaluation of DED subtypes and their treatment strategies.

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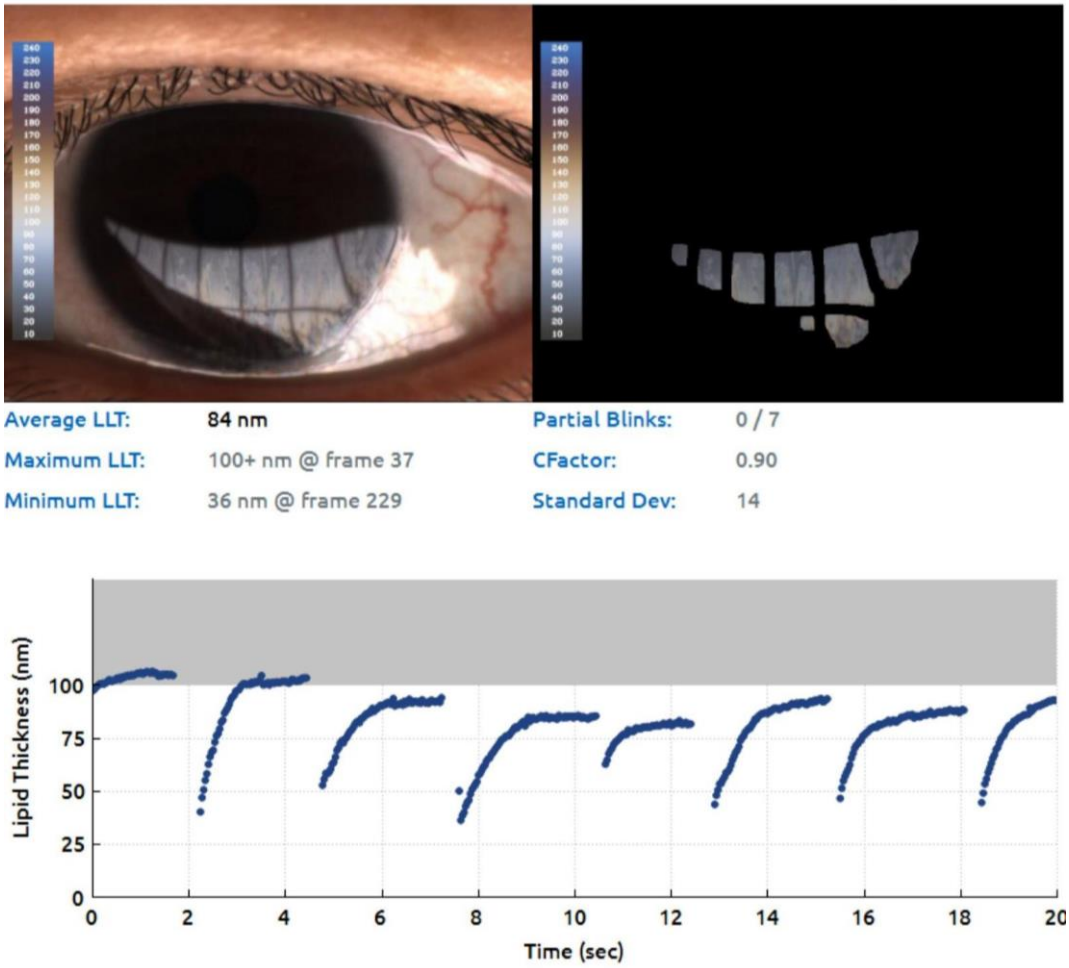
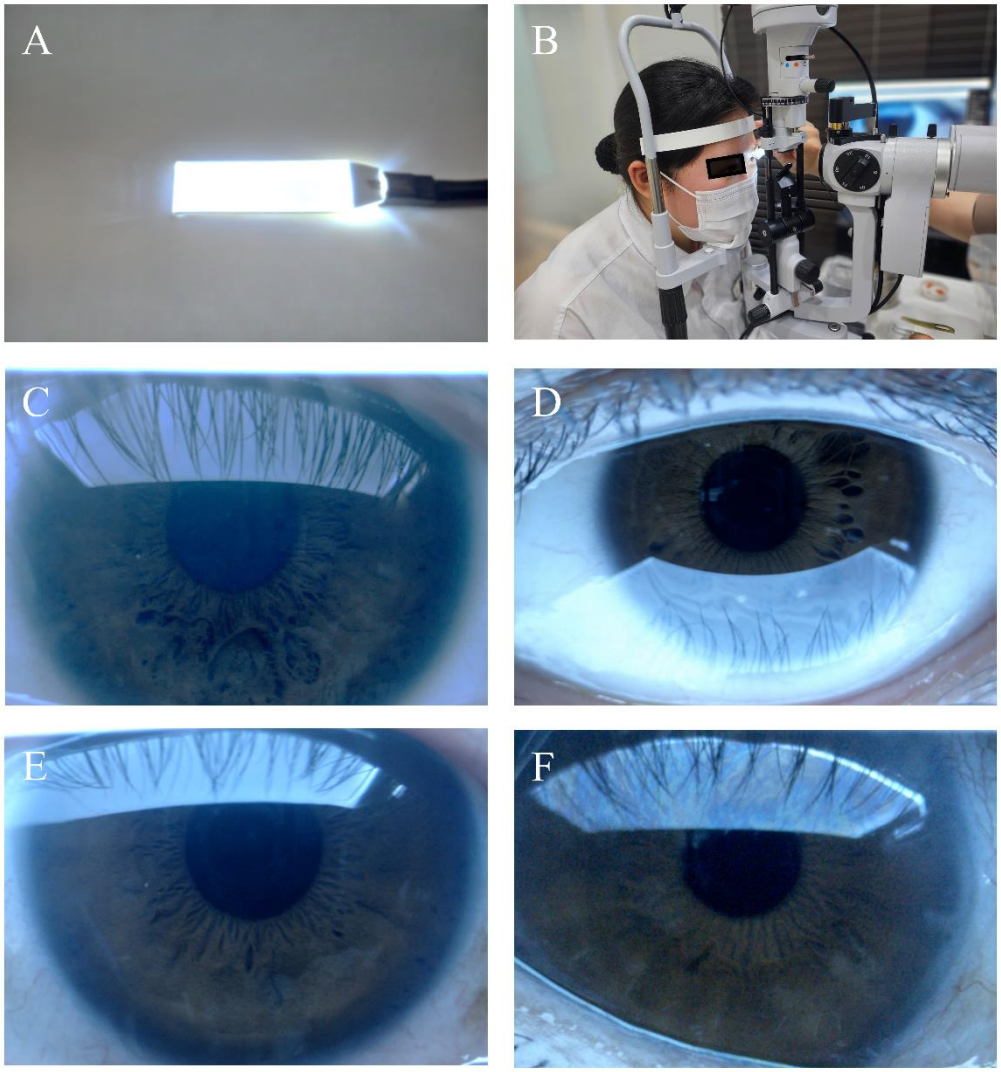
APPENDICES 1.



Appendices 1. Example of a tear interferometry report generated by the TearScience™ LipiView® II Ocular Surface Interferometer (Johnson & Johnson Vision, Jacksonville, Florida, United States). The device automatically records 20-second videos reflecting lipid layer thickness (LLT) on the inferior cornea and generates a graphical summary display. The quantified LLT is measured over time in interferometric color units (ICU), where one ICU corresponds to approximately 1 nm of LLT.

APPENDICES 2.



Appendices 2. Device (A, B) and examples (C, D, E, F) of manual lipid layer thickness (LLT) measurement. (A) Whitish LED plate used for measuring superior corneal LLT in this study. (B) Measurement performed under slit-lamp examination, followed by anterior segment photography. The illumination pattern of the superior cornea was classified into three grades using a modified version of a previously established system.[14] (C) A dark, uniform pattern, classified as Level 0. (D) A gray, non-uniform pattern, classified as Level 1. (E) A gray, uniform pattern, classified as Level 1. (F) A colored, non-uniform pattern, classified as level 2.

APPENDICES 3.

Appendices 3. The intra-/interclass correlation coefficient of the superior corneal lipid layer thickness (LLTsup) and fluorescein tear break-up pattern (FTBUP) measurements

LLT_{sup}	Fleiss κ	95% confidence interval	
		Lower	Upper
Grade L (dark, uniform)	0.731	0.525	0.938
Grade M (gray, uniform and non-uniform)	0.679	0.472	0.885
Grade H (colored, non-uniform)	0.949	0.743	1.156
Total	0.791	0.642	0.941

FTBUP	Fleiss κ	95% confidence interval	
		Lower	Upper
Dimple break	0.520	0.313	0.727
Spot break	1.000	0.793	1.207
Random break	0.750	0.430	0.844
Line break	0.850	0.601	1.014
Area break	1.000	0.793	1.207
Total	0.772	0.666	0.879

Three independent examiners assessed manual tear interferometry images and fluorescein tear break-up videoclips. All 310 study subjects were evaluated, and the most favored classification was used in the study.

APPENDICES 4.

Appendices 4. The Meibomian Gland Evaluator (Left, Johnson & Johnson Vision, Jacksonville, Florida, United States). Meibomian gland expression was measured at a pressure of 0.3 pounds per square inch (approximately 15 mmHg) using the Meibomian Gland Evaluator in the central 8 glands of the upper and lower eyelids.

APPENDICES 5.

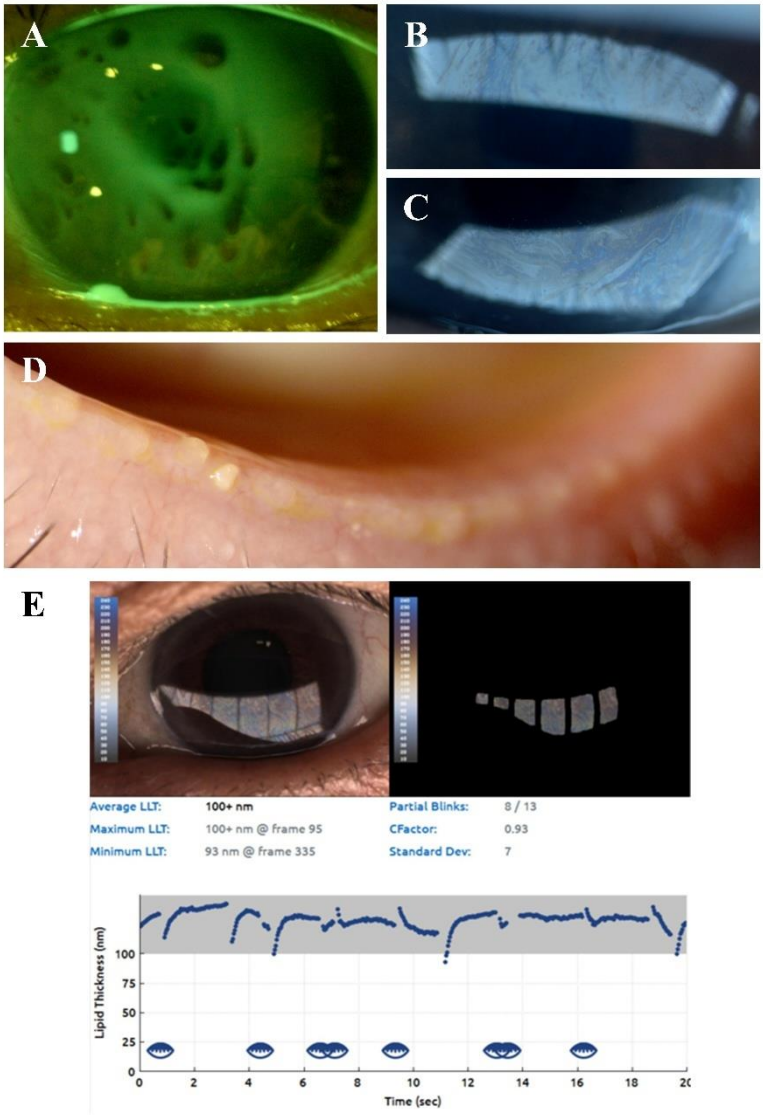
Appendices 5. Characteristics of study subjects.

Characteristics	Units	Values
Age	years, Mean ± SD	50.0 ± 15.0
Sex	N, % of female	220, 71.0
Conventional DED assessments		
OSDI	scores, Mean ± SD	28.1 ± 12.6
TBUT	seconds, Mean ± SD	2.6 ± 1.9
NIKBUT	seconds, Mean ± SD	5.6 ± 2.7
CSS	scores, Mean ± SD	0.5 ± 1.1
Aqueous assessments		
TMH	μm, Mean ± SD	225.5 ± 45.9
Schirmer I test	mm, Mean ± SD	7.6 ± 3.9
Lipid assessments		
MGE	grades, Mean ± SD	1.3 ± 0.9
Grade 0 (none)	N, %	64, 20.6
Grade 1 (minimal to mild)	N, %	125, 40.3
Grade 2 (moderate)	N, %	87, 28.1
Grade 3 (severe)	N, %	34, 11.0
MQ	grades, Mean ± SD	2.1 ± 0.8
Grade 0 (clear)	N, %	12, 3.9
Grade 1 (cloudy)	N, %	74, 23.9
Grade 2 (cloudy particulate)	N, %	86, 27.7
Grade 3 (toothpaste or no secretion)	N, %	138, 44.5
Lid margin abnormality		
Plugging	N, %	50, 16.1
Telangiectasia	N, %	39, 12.6
Anterior shift of MCJ	N, %	102, 32.9
Notching	N, %	56, 18.1
Epithelial desquamation	N, %	79, 25.5
Tear interferometric lipid layer thickness		
LLT_{inf}	nm, Mean ± SD	78.7 ± 31.3
Grade L (LLT_{inf} <40 nm)	N, %	70, 22.6
Grade M (40 nm ≤ LLT_{inf} < 90 nm)	N, %	119, 38.4
Grade H (LLT_{inf} ≥ 90nm)	N, %	121, 39.0

LLT_{sup} grade		
Grade L (dark, uniform)	N, %	135, 43.5
Grade M (gray, uniform and non-uniform)	N, %	105, 33.9
Grade H (colored, non-uniform)	N, %	70, 22.6
Fluorescence tear break-up patterns		
Dimple break	N, %	34, 11.0
Spot break	N, %	99, 31.9
Random break	N, %	98, 31.6
Line break	N, %	72, 23.2
Area break	N, %	7, 2.3

Abbreviations: CSS, corneal staining score; DED, dry eye disease; LLT, lipid layer thickness; MCJ, mucocutaneous junction; MGE, meibomian gland expressibility; MQ, meibum quality; NIKBUT, non-invasive keratography tear break-up time; OSDI, ocular surface disease index; TBUT, fluorescence tear break-up time; TMH, tear meniscus height.

APPENDICES 6.



Appendices 6. A case example of 66-year-old female patient. The left eye of the patient presented with a tear meniscus height of 209 μm, and no signs of corneal erosion were observed. The fluorescein tear break-up pattern indicated a "spot break" type with a tear break-up time of under 1 second, occurring immediately after the up-motion of the upper eyelid (A). Both the inferior and superior cornea showed a high lipid layer thickness (LLT) with a colored, non-uniform pattern (B, C). After eyelid squeezing, meibomian gland expressibility was graded as 0, and meibum quality was graded as 1 (D). The inferior LLT, measured using the LipiView® II Ocular Surface Interferometer, was 112 nm (E).

ABSTRACT IN KOREAN

눈물 간섭계를 이용한 건성안 아형 평가

본 연구는 눈물 간섭계를 활용하여 눈물 역학을 분석하고, 이를 통해 건성안 아형(마이봄샘기능이상, 수분결핍)을 평가하는 데 있어 지질층 두께의 역할과 진단적 유용성을 조사하는 것을 목표로 한다. 건성안 환자를 대상으로 단면 연구를 수행하였으며, 하부 및 상부 각막의 지질층 두께(LLT_{inf}, LLT_{sup})를 측정하였다. 안구표면질환지수 (Ocular Surface Disease Index), 눈물띠 높이, 마이봄샘 분비 기능 정도(meibomian gland expressibility), 각막 염색 점수 (corneal staining score) 등 다양한 안구건조증 임상지표와의 연관성을 총 310명의 310안을 대상으로 분석하였다. 지질층 두께는 건성안의 병태생리에 따라 다양한 패턴을 보였으며, LLT_{inf}와 LLT_{sup}를 함께 고려할 경우 수분결핍 예측에 대한 정확도가 증가하였다. LLT_{inf}가 낮을수록 마이봄샘기능이상이 증가하였다. LLT_{inf}가 높은 경우, LLT_{sup}에 따라 안구건조증 아형의 분포가 달라지는데, LLT_{sup}가 낮을수록 수분결핍 아형이 두드러지는 것으로 나타났다. 결론적으로, 눈물 간섭계를 이용한 눈물 지질층 두께 측정은 안구건조증의 아형을 평가하는 데 유용한 도구가 될 수 있으며, 특히 상하부 지질층 두께를 종합적으로 분석할 경우 보다 정밀한 진단이 가능하며, 개별 환자의 눈물의 역학을 평가하는데 도움이 될 것으로 사료된다.

핵심되는 말: 눈물 간섭계, 눈물막 기반 진단, 눈물 지질층 두께, 마이봄샘기능장애, 수분결핍 안구건조증