BMC Ophthalmology

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Effect of lid-wiper epitheliopathy on the corneal epithelial thickness distribution: a novel semiautomatic quantitative method

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Abstract

Background To explore a novel semi-automatic quantitative method for detecting lid-wiper epitheliopathy (LWE) and analyze its impact on the distribution of corneal epithelial thickness (CET).

Methods A total of 37 eyes from 37 adults were included in this study. The lid wipers were stained with lissamine green strips, and the stained areas were recorded using a digital slit-lamp biomicroscopy and analyzed using ImageJ software. Factors such as age, sex, and refraction were included as variables that influenced LWE. The CET was divided into 25 regions and recorded using spectral-domain optical coherence tomography.

Results Methodological consistency and repeatability were verified through intra- and inter-operator intraclass correlation coefficients, with values of 0.889 and 0.922, respectively. After adjusting for age, sex, and spherical equivalent refraction, a negative correlation was observed between LWE and epithelial thickness. Specifically, negative correlations were observed in the nasal, subnasal, and inferior regions within the 2–5 mm range; subnasal and infratemporal regions within the 5–7 mm range; and the infratemporal region within the 7–9 mm range.

Conclusions This study validated a novel method for assessing the severity of LWE by quantitatively analyzing the LWE area. Additionally, LWE is associated with thinning of the inferior corneal epithelium.

Keywords Corneal epithelial thickness, Lid-wiper epitheliopathy, Semiautomatic quantitative method

Background

The corneal epithelial thickness (CET) is unevenly distributed, with a tendency for greater thickness in the inferior and nasal regions [1–4]. Reinstein [5] reported that blinking and rubbing of the cornea may regulate CET distribution. The interaction between the upper lid wiper and the ocular surface is the primary source of friction during the normal blinking process. The lid wiper (LW) concept was first described by Korb [6], which is a part of the conjunctival edge and plays a crucial role in the blinking process, ensuring even distribution of the tear film across the ocular surface. The lid-wiper of the upper eyelid exhibits predominant vertical movements during blinking and has gained considerable attention [7].

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Lid wiper epitheliopathy (LWE) is defined as damage to the epithelium of the LW, primarily caused by insufficient lubrication of the ocular surface for various reasons (such as inadequate interface lubrication, uneven corneoconjunctival surface, abnormal blinking pattern, and frequency), leading to increased friction between the LW and the ocular surface [8–10]. This indicates that LWE may be a potential factor contributing to the uneven distribution of CET, and this aspect has been consistently overlooked in previous studies [11–13].

The conventional method for assessing LWE involves initial staining with lissamine green, followed by the examiner's evaluation of the extent of damage in both horizontal and vertical dimensions. The mean of these ratings was calculated to determine the final grade [6, 14–16]. Kunnen [17] introduced a new grading method that quantitatively analyzed the length and width of the LWE staining area before assigning grades. This approach demonstrated that traditional subjective grading frequently underestimated staining width and overestimated staining length. However, this method has several limitations. Because the stained area of LWE is often irregular and sometimes discontinuous, merely assessing its length and width cannot accurately reflect the actual size of the affected area.

Therefore, this study aimed to propose a novel semi-automatic quantitative method based on the stained area of LWE to reflect the severity of LWE more accurately and subsequently explore its correlation with CET distribution.

Methods

Patients

This cross-sectional study included 37 healthy adults (37 eyes) who were examined at the Affiliated Ophthalmology Hospital of Wenzhou Medical University. Only data from the right eye were used for the statistical analysis. The study protocol and the informed consent form were approved by the hospital ethics committee board (approval no. 2019-228-K-203-1) and complied with the tenets of the Declaration of Helsinki.

Prior to the study, all participants were fully informed of the research content and potential risks involved, and written informed consent was obtained. The inclusion criteria were as follows: (1) Presence of no apparent abnormalities in routine ophthalmic examinations, including visual acuity, refraction, intraocular pressure, slit-lamp, and fundoscopic examinations; and (2) LWE can be successfully stained. Patients with a history of contact lens use, eyelid diseases (such as blepharoptosis, lagophthalmos, blepharospasm, entropion, or ectropion), or previous ocular surgery were excluded.

Examinations and measurements

All patients underwent a thorough ophthalmic examination that included measurement of intraocular pressure with a noncontact tonometer (TX-20P, Canon, Japan), manifest refraction, slit-lamp examination, and fundus examination.

CET was examined using SD-OCT (RTVue-XR, Optovue, Inc., Fremont, CA, USA) at least 4 h after the participants awakened from sleep. The collected data were processed and analyzed using the system software. The CET of the following four areas was recorded: (1) the central area of the cornea with a diameter within 2 mm. (2) Eight paracentric regions within the range of 2–5 mm. (3) Eight central peripheral regions within the range of 5–7 mm. (4) Eight peripheral areas within the range of 7–9 mm. The eight regions located in the paracentric, central peripheral, and peripheral regions were the superior, supranasal, nasal, subnasal, inferior, infratemporal, temporal, and supratemporal regions. The calculation of differences in epithelial distribution involves subtracting the superior from the inferior region, the supratemporal from the infratemporal region, the supranasal from the subnasal region, and the temporal from the nasal region.

The standard staining method with GreenGlo, a lissamine green strip (HUB Pharmaceuticals, LLC, USA), was used in this study to demonstrate LWE [6, 14, 18]. The strip was infiltrated with one drop of sodium hyaluronate to ensure even distribution. After pulling the lower eyelid, the strip tip was brought into contact with the lower tarsal conjunctiva for staining. Following the initial staining, patients were instructed to wait for 5 min with their eyes closed before the next instillation. Images of the lissamine green staining were captured 1 min after the second instillation. The examiner then used a digital slit-lamp biomicroscope (Chongqing Kanghua Ruiming Technology Co., Ltd., China) with a 6x lens and a frosted glass sheet at maximum brightness to observe and record lissamine green staining in the LW area. To prevent iatrogenic staining during the flipping process, the examiner held the base of the right eyelash and the surface of the upper eyelid to create an ectropion of the right upper eyelid while ensuring that the LW was not in contact. Three photographs were taken for each eye, and the best one was selected for analysis. A calibration length of 1 cm was used for each photograph.

The traditional subjective grading was performed by two experts. Following the method proposed by Korb [6], the assessment was conducted based on two dimensions: the width and height of the LWE. The final grade of the LWE was determined by taking the average of the grades for both dimensions. The mean of the grades provided by the two experts was then included in the statistical analysis.

The ImageJ software (ver. 1.52a) was used to process and calculate the images captured during the examination. The examiner set the scale according to the calibration length of 1 cm, and the "Polygon Tool" was used to draw the area of the LW and LWE at high magnification (Fig. 1). The software automatically calculates the selected area. The ratio of the LWE area to the LW area ($Ratio_{LWE/LW}$) was calculated manually.

All the measurements were performed by a single examiner. Image processing and analysis were performed by two experts, and each image was analyzed three times at different time intervals. To verify the repeatability of this experimental method, two datasets were selected from an individual expert's analyses at different periods. To verify the consistency of the experimental methods, both experts analyzed the identical group of images.

Statistical analysis

All statistical analyses were performed using SPSS version 26 software (IBM Corp., Armonk, NY, USA). The normality of the data distribution was examined, and the results are presented as the mean ± SD and medians and quartiles for normally and non-normally distributed data, respectively. The within-subject standard deviation (Sw), test-retest repeatability (TRT), coefficient of variation (CoV), intraclass correlation coefficient (ICC), and Bland–-Altman plot were used to assess the repeatability and consistency of the proposed method. TRT was defined as 2.77 times SW, and a smaller value for both SW and TRT indicated better measurement repeatability. An ICC $>$ 0.8 and a CoV$<$ 10% indicated good repeatability for all parameters. Spearman correlation analysis was used to assess the correlation between LWE and CET distribution, as well as between LWE and the difference in epithelial distribution. Additionally, generalized estimating equations (GEE) were used to adjust the correlation analysis results, with adjustment factors including age, gender, and spherical equivalent refraction (SER). Statistical significance was set at $P < 0.05$.

Results

Basic eye health examination

A total of 37 eyes from 37 healthy individuals (comprising 21 (56.8%) men and 16 (43.2%) women; mean age 23.51 ± 3.99 years) were included in this cross-sectional study. The mean intraocular pressure was 15.05 ± 2.19 mmHg, the SER was − 5.79 ± 3.06 D, and all corrected visual acuity was ≤ 0 LogMAR. All patients underwent 90D fundus examination under mydriasis, and the results were normal. No obvious complications were reported after the successful completion of the procedure.

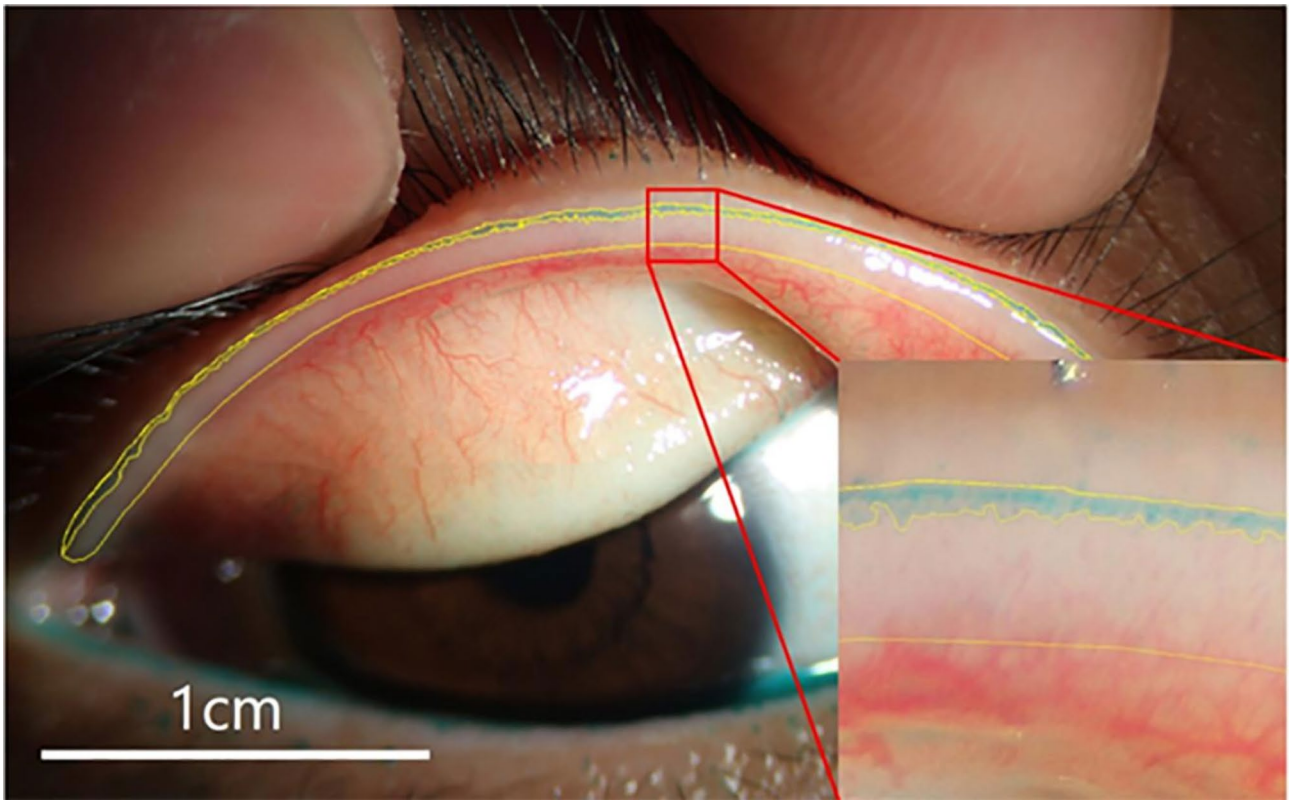

Fig. 1 Illustration after image processing. The areas marked with yellow lines are the LW area and the LWE area. The picture in the lower right corner is a magnified picture of the red area. The length of the marking is 1 cm

Repeatability, consistency, and influencing factors of LWE measurements

The traditional subjective grade for LWE is 1.5(1.0,2.25). The upper LWE area observed in this study was 0.089 (0.078, 0.130) cm^2, while the LW area was 0.335 ± 0.010 cm^2 and the $Ratio_{LWE/LW}$ was 0.331 (0.300,0.357). The subjective grade showed a strong correlation with both the LWE area ($r = 0.818$, $P < 0.001$) and the $Ratio_{LWE/LW}$ ($r = 0.855$, $P < 0.001$). The intra-operator repeatability indices, including an ICC of 0.889, Sw of 0.007, TRT of 0.019, and CoV of 8.84%, indicated excellent repeatability. The ICC for inter-operator consistency was 0.922. As shown in Fig. 2, the points are clustered closely around the mean difference, indicating a good agreement with minimal systematic bias. The 95% limits of agreement, represented by brown dashed lines, suggest that the majority of the differences are within an acceptable range, confirming the good reproducibility of the measurements.

The GEE indicated that age (B = 0.002, $P = 0.265$), sex (B=-0.014, $P = 0.384$), and SER (B=-0.001, $P = 0.666$) were not independent factors influencing LWE.

Correlation between LWE and the CET distribution

The CET distribution demonstrated superior thinning, inferior thickening, nasal thinning, and temporal thickening patterns (Table 1).

Although LWE and CET distribution were not correlated in Spearman's correlation analysis, GEE analysis following adjustments for age, sex, and SER revealed a negative correlation between LWE and epithelial thickness. In particular, negative correlations were observed in the nasal, subnasal, and inferior regions within the 2–5 mm range; subnasal and infratemporal regions within the 5–7 mm range; and the infratemporal region within the 7–9 mm range (Table 1; Fig. 3). On the contrary, no correlation was found between CET distribution and $Ratio_{LWE/LW}$ (Appendix Table 1).

Furthermore, this study explored the correlation between LWE and the differences in corneal thickness between the upper and lower regions. A negative correlation was observed between LWE and differences in the 2–5 mm range for the superior and inferior regions; subnasal and supranasal differences; infratemporal and supratemporal differences; subnasal and supranasal differences in the 5–7 mm range; infratemporal and supratemporal differences; and subnasal and supratemporal differences in the 7–9 mm range (Table 2; Fig. 4). Similar results were revealed between the $Ratio_{LWE/LW}$ and the difference in CET distribution (Appendix Table 2).

Discussion

The method employed in this study comprised two crucial steps: staining and quantitative analysis. Previous studies have indicated that the dye concentration, volume, and time to contact the eye surface are crucial factors in determining the degree of staining [19]. To ensure successful and thorough staining of the LWEs, the staining procedure employed in this study complied with the recommendation in the latest TFOS DEWSII report [20], and the GreenGlo strips used in this study were proven to yield the most effective staining results [21].The strong repeatability and consistency of the method was validated during the quantitative analysis process using ImageJ software to outline the LWE regions and automatically measure their areas. This method, as opposed

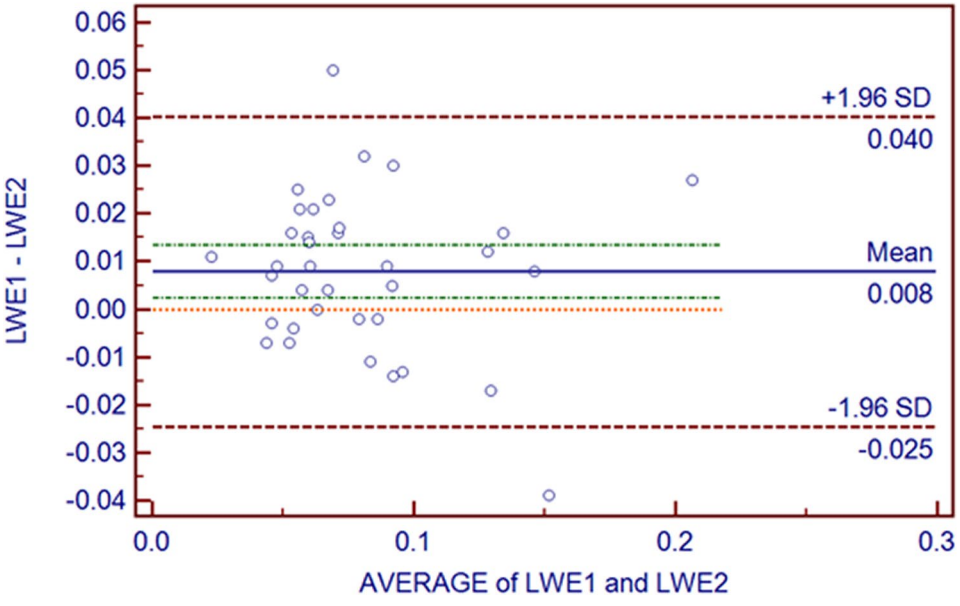

Fig. 2 Bland–Altman plot of the results of image analysis performed by two examiners. The 95% limits of agreement, represented by brown dashed lines

Table 1 Analysis of the correlation between the upper LWE area and the distribution of CET

Area		CET	Upper LWE area(Spearman)		Upper LWE area(GEE)	
			r	*P* value	B	*P* value
Central cornea		53.43 ± 2.95	-0.180	0.288	-19.118	0.057
2–5 mm Paracentric	superior	52.00 ± 3.10	0.120	0.480	0.468	0.963
	supranasal	52.65 ± 3.21	0.003	0.985	-8.555	0.392
	nasal	53.70 ± 3.14	-0.202	0.231	-22.862	0.026*
	subnasal	54.51 ± 3,19	-0.263	0.116	-27.342	0.009*
	inferior	54.46 ± 3.21	-0.226	0.178	-24.255	0.024*
	infratemporal	54.14 ± 3.11	-0.189	0.262	-19.245	0.068
	temporal	53.57 ± 2.85	-0.013	0.941	-9.891	0.347
	supratemporal	52.65 ± 2.91	0.097	0.567	-3.785	0.717
5–7 mm Central peripheral	superior	49.30 ± 3.41	0.041	0.810	-1.287	0.937
	supranasal	51.38 ± 3.47	0.088	0.604	-3.910	0.744
	nasal	53.49 ± 3.18	-0.164	0.332	-18.457	0.087
	subnasal	54.08 ± 3.02	-0.231	0.170	-19.630	0.049*
	inferior	54.08 ± 3.17	-0.150	0.377	-13.404	0.177
	infratemporal	53.78 ± 2.96	-0.239	0.154	-22.691	0.018*
	temporal	53.41 ± 3.05	0.040	0.814	-7.189	0.501
	supratemporal	51.49 ± 3.27	0.201	0.232	1.125	0.920
7–9 mm Periphery	superior	43.62 ± 3.71	0.148	0.381	19.653	0.219
	supranasal	47.41 ± 3.77	0.069	0.686	8.006	0.600
	nasal	53.11 ± 3.14	-0.158	0.349	1.775	0.183
	subnasal	53.00 ± 3.17	-0.105	0.534	-8.839	0.367
	inferior	52.68 ± 3.38	-0.103	0.544	-4.419	0.669
	infratemporal	53.00 ± 3.17	-0.280	0.093	-20.304	0.016*
	temporal	52.22 ± 2.83	0.002	0.993	-8.629	0.330
	supratemporal	48.00 ± 3.56	0.243	0.147	12.687	0.303

* indicates statistical significance, $P < 0.05$

to conventional analysis based solely on the length and width of the LWE regions [17], maximally retains comprehensive information of the LWE area. In addition, it mitigates potential errors stemming from subjective grading [6, 14–16].

Consistent with previous findings, this study revealed no significant effects of sex [22, 23], age [24], or refractive error on LWE [25]; however, several studies have identified age as a factor that influences LWE. This could be attributed to the variation in age of the study participants. The age range of the study population in these two studies was 19–70 years [22] and 3–94 years [23], whereas the participants in this study were relatively concentrated in the age range of 18–37 years.

As previously described [1], the CET distribution in this study displayed a thinner pattern superiorly and nasally and thicker at the inferior and temporal regions. Interestingly, notable thinning of the corneal epithelium in the inferior region was observed with increasing LWE severity, with a relatively minor impact on the superior epithelium. Concurrently, the difference in thickness between the superior and inferior corneal epithelium decreased. Using a microscope, Mathers et al. [26] observed that the shearing force during the blinking process led to the shedding of corneal epithelial cells, subsequently altering their distribution and renewal. In this study, the increased friction caused by LWE may have resulted in the thinning of the corneal epithelium. Jones et al. [27] reported that at the lifting phase of blinking, the force exerted by the eyelids on the surface of the eyeball was significantly greater than that at the stretching phase of blinking. During the eyelid lifting process, based on the vector decomposition of the applied force, the force exerted on the lower cornea is greater than that on the upper cornea, which could contribute to the more pronounced changes in the thickness of the inferior corneal epithelium.

Detecting a thinner corneal epithelial thickness in individuals with asymptomatic LWE could have been a sign of subclinical ocular surface stress or early-stage corneal dysfunction. While these individuals may not have experienced symptoms at the time, the findings could have indicated an increased risk of developing dry eye disease, corneal damage, or other ocular surface disorders in the future, making them candidates for closer monitoring and preventive care.

This study had the following limitations: First, the sample size was relatively small, and the participants were mainly selected from a population undergoing preoperative examination at a refractive center, resulting in a

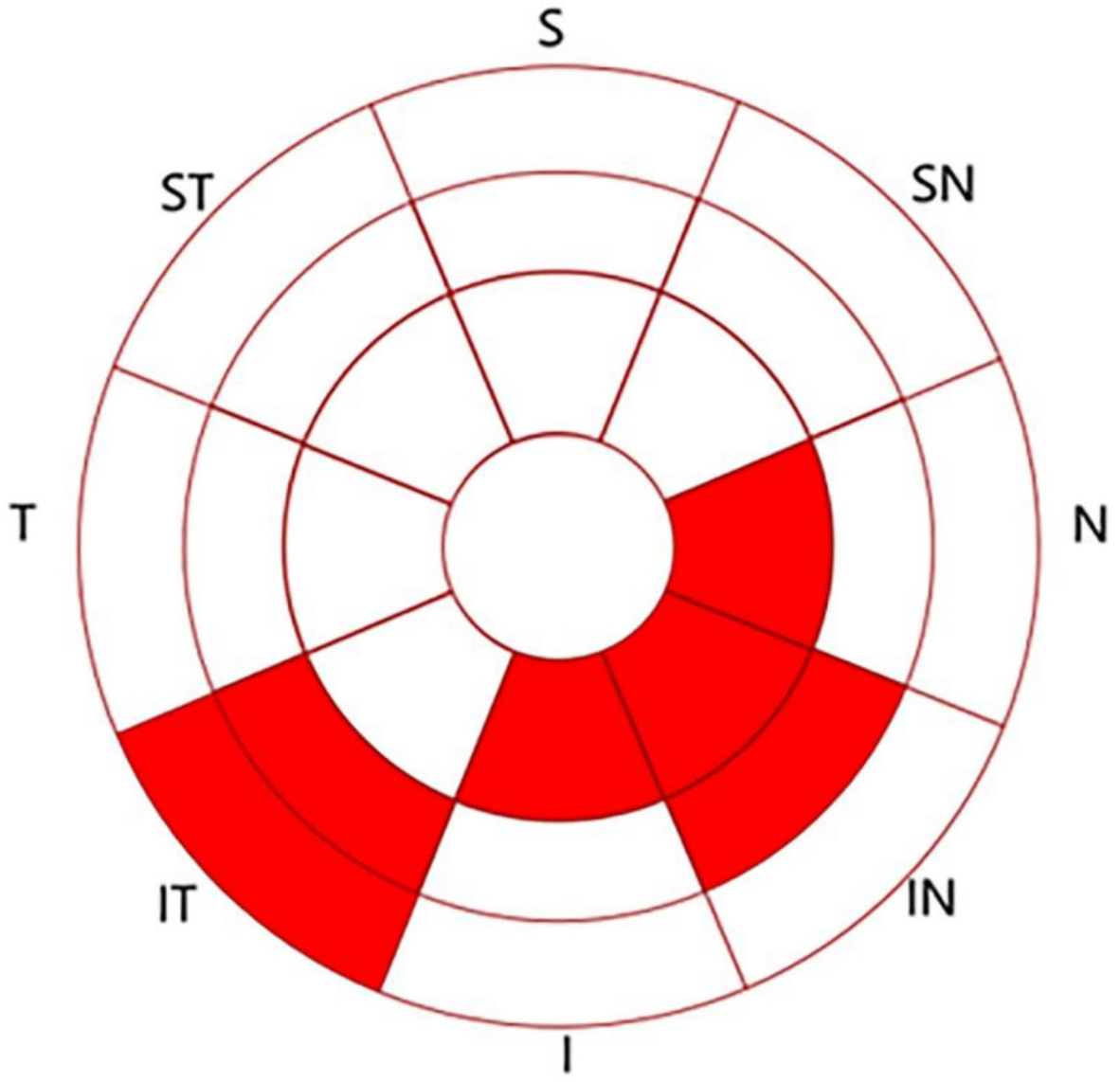

Fig. 3 Correlation between the upper LWE area and the distribution of CET. The concentric circles from inside to outside are the central cornea, the paracentric region, the Central peripheral region and the peripheral region. Red areas indicate a negative correlation between the upper LWE area and the distribution of CET

concentration of age. Second, although the agreement between the two examiners was good, the use of the ImageJ software for the digital analysis of images may be time-consuming for routine clinical use and may be more suitable for research purposes. Third, manual identification is required to determine the stained areas, which may have introduced artificial errors.

Conclusions

This study validated a novel method for assessing the severity of LWE through quantitative analysis of the LWE area and demonstrated excellent repeatability and consistency. No significant effects of age, sex, or refraction on the LWE were observed. Building on this foundation, it was discovered that LWE is associated with thinning of the inferior corneal epithelium. Consequently, the influence of LWE should be considered when investigating corneal epithelial distribution.

Table 2 Correlation analysis of the upper LWE area and the difference in CET distribution

	Region	Upper LWE area(Spearman)		Upper LWE area(GEE)	
		r	P value	B	P value
2–5 mm Paracentric	inferior-superior	-0.564	< 0.001	-23.832	< 0.001
	infratemporal- supratemporal	-0.453	0.005	-14.853	0.002
	subnasal- supranasal	-0.453	0.005	-18.051	0.002
	nasal-temporal	0.223	0.185	8.250	0.270
5–7 mm Central peripheral	inferior-superior	-0.201	0.233	-12.703	0.302
	infratemporal- supratemporal	-0.466	0.004	-23.740	< 0.001
	subnasal- supranasal	-0.344	0.037	-15.828	0.003
	nasal-temporal	-0.239	0.154	-12.133	0.113
7–9 mm Periphery	inferior-superior	-0.221	0.188	-25.807	0.127
	infratemporal- supratemporal	-0.377	0.021	-33.513	0.001
	subnasal- supranasal	-0.342	0.039	-24.219	< 0.001
	nasal-temporal	-0.184	0.275	-7.853	0.302

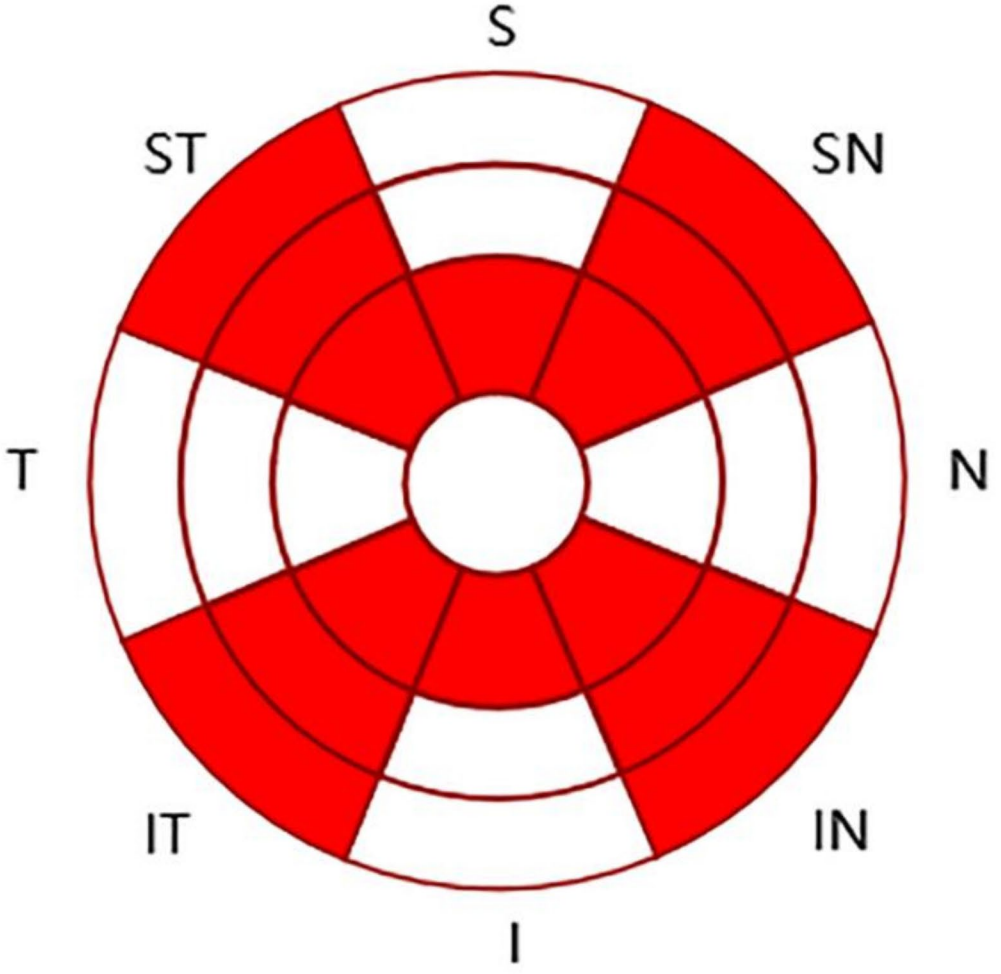

Fig. 4 Correlation between the upper LWE staining area and the difference in CET distribution. The concentric circles from inside to outside are the central cornea, the paracentric region, the Central peripheral region and the peripheral region. Red areas indicate a negative correlation between the upper LWE staining area and the difference in CET distribution

Abbreviations

CET	Corneal epithelial thickness
LW	Lid wiper
LWE	Lid wiper epitheliopathy
Sw	Within-subject standard deviation
TRT	Test-retest repeatability
CoV	Coefficient of variation
ICC	Intraclass correlation coefficient
SER	Spherical equivalent refraction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12886-025-03918-y.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
Not applicable.

Author contributions
Study conception and design (JJY, ML and LH); Data acquisition and analysis (JJY, DM, ZYL); Manuscript writing (JJY, ML, JL); Manuscript polishing (XZC, YHY) Critically revising the manuscript for intellectual content: (LH). All authors made final approval of the version to be published and agreed to be accountable for all aspects of the work.

Funding
This work was funded by the Science Technology Department of Zhejiang Province, LGF22H120014, 2023C03106,the Wenzhou Science and Technology Bureau, ZY2020010.

Data availability
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Prior to the study, all participants were fully informed of the research content and potential risks involved, and written informed consent was obtained. The study protocol and the informed consent form were approved by the Ethics Committee of Eye Hospital of Wenzhou Medical University, which belongs to Eye Hospital of Wenzhou Medical University (approval no. 2019-228-K-203-1) and complied with the tenets of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 30 April 2024 / Accepted: 12 February 2025
Published online: 07 March 2025

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