• Clinical Research •

Correlating corneal and meibomian gland morphology with diabetes duration in T2DM *via* confocal microscopy

Ke–Jun Li[1], *Yin Zhang*[1], *Yi–Ze Han*[2], *Zhi–Hua Zhao*[1], *Fang Fan*[1], *Yi–Ming Chen*[1], *Jian–Min Wang*[1]

[1]Department of Ophthalmology, Hebei Provincial People's Hospital, Shijiazhuang 050051, Hebei Province, China
[2]Department of Ophthalmology, Shijiazhuang Municipal People's Hospital, Shijiazhuang 050000, Hebei Province, China

Correspondence to: Jian-Min Wang. Department of Ophthalmology, Hebei Provincial People's Hospital, No.348, Heping West Road, Shijiazhuang 050051, Hebei Province, China. wjmykys@163.com

Received: 2025-05-21 Accepted: 2026-03-26

Abstract

● **AIM:** To investigate morphological changes in the cornea and meibomian glands using *in vivo* confocal microscopy (IVCM) in patients with type 2 diabetes (T2DM) and to analyze the correlation of these changes with disease duration (DD).

● **METHODS:** Patients with T2DM who visited the ophthalmology and endocrinology departments of our hospital from May 2023 to December 2023 were selected. According to DD, they were divided into the short-DD (≤10y) and long-DD (>10y) groups. The control group consisted of age-matched non-diabetes patients. All underwent Ocular Surface Disease Index (OSDI), tear break-up time (TBUT), Schirmer I test (SIT), and IVCM imaging. Corneal nerve parameters, including corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), and meibomian gland metrics, including density of meibomian gland acinar units (MGAUD), the longest diameter of the meibomian gland acini (MGALD), the shortest diameter of meibomian gland acini (MGASD), the area of meibomian gland acinar units (MGAUA), were analyzed using ImageJ and ACCMetrics.

● **RESULTS:** A total of 130 patients with T2DM were included in this study, among which 68 were male (52.3%, age 30-76y) and 62 were female (47.7%, age 30-76y), the average age of the group was 55.54±11.65y. Significant differences (P<0.001) were observed in OSDI, TBUT, SIT, CNFD, CNBD, CNFL, MGALD, MGASD, MGAUD, and MGAUA between diabetes groups and controls. DD positively correlated with OSDI, MGALD, and MGASD (P<0.05), and negatively correlated with TBUT, SIT, CNFD, and CNFL (P<0.05). No correlation was found between DD and CNBD, MGAUD, or MGAUA (P>0.05).

● **CONCLUSION:** T2DM patients exhibit greater corneal nerve and meibomian gland damage than age 30-76y non-type 2 diabetes patients. Prolonged DD exacerbates these morphological changes.

● **KEYWORDS:** diabetes; corneal nerves; meibomian glands; *in vivo* confocal microscopy; ocular surface

DOI:10.18240/ijo.2026.06.16

Citation: Li KJ, Zhang Y, Han YZ, Zhao ZH, Fan F, Chen YM, Wang JM. Correlating corneal and meibomian gland morphology with diabetes duration in T2DM *via* confocal microscopy. *Int J Ophthalmol* 2026;19(6):1149-1157

INTRODUCTION

Type 2 diabetes comprises more than 90 percent of all diabetic cases and is commonly linked to the traits of metabolic syndrome, such as abdominal obesity, hypertension, insulin resistance, and dyslipidemia. Although this form of diabetes has long been associated with microvascular complications including retinopathy, nephropathy, and peripheral neuropathy, emerging evidence continues to underline its extensive systemic implicationst[1]. The prevalence of type 2 diabetes is steadily increasing, with an increase of 200 million cases projected by 2040. By 2045, the global number of cases is estimated to reach 783 million[2]. Medical personnel's understanding of the ocular complications of type 2 diabetes should not be limited to diabetic retinopathy, macular edema, metabolic cataracts, and neovascular glaucoma; entire ocular surface disorders similarly affect individuals' quality of life and can seriously threaten vision. Common ocular surface disorders such as meibomian gland dysfunction (MGD), dry eye, and keratopathy have been shown in studies to affect as many as 70% of patients with type 2 diabetes[3], with the incidence of dry eye ranging between 27.7% and 54.3%[4]. With the advancements in ophthalmic examination equipment in recent years, more and more scholars have discovered that *in vivo* confocal microscopy (IVCM) plays a crucial role in diagnosing corneal and meibomian gland diseases. Beyond

ocular diseases, IVCM functions as a surrogate biomarker for systemic diseases by assessing the density, length, and branch density of corneal nerve fibers. IVCM is a noninvasive scanning modality that provides detailed information on the corneal nerve plexus and has become a valuable tool and the gold standard for ophthalmologists to evaluate the status of corneal nerve fibers[5-6]. This study utilizes IVCM to explore the abnormalities in the morphology of corneal and meibomian gland tissues in patients with type 2 diabetes and analyzes their correlation with the duration of diabetes, providing guidance for the early diagnosis and treatment of diabetic superficial eye diseases.

PARTICTPANTS AND METHODS

Ethical Approval This study followed the Declaration of Helsinki and was reviewed and approved by the Medical Ethics Committee of Hebei General Hospital. Informed consent was obtained from all participants. Ethics review number: (2024) (094).

Participants This study selected a total of 130 patients with type 2 diabetes who visited the ophthalmology and endocrinology departments of Hebei Provincial People's Hospital from May 2023 to December 2023. According to the duration of diabetes[7], they were divided into a short-duration group (diabetes duration ≤10y) with 67 cases, and a long-duration group (diabetes duration >10y) with 63 cases. The control group consisted of 20 non-type 2 diabetes patients who visited the same ophthalmology department during the same period.

Inclusion Criteria Diabetes group: 1) age ≥18y, gender unspecified; 2) patients diagnosed with type 2 diabetes by the Endocrinology Department of Hebei Provincial People's Hospital; 3) with mild non-proliferative diabetic retinopathy or without any diabetic retinopathy. Healthy control group: 1) age ≥18y, regardless of gender; 2) patients without diabetes; 3) no MGD, according to the criteria by Bron *et al*[8], diagnosis of MGD included one or more of the following: absent, viscous, or waxy white secretion on digital expression, presence of greater than two lid margin telangiectases, and plugging of greater than two gland orifices; 4) voluntarily participating in this study.

Exclusion Criteria 1) age <18y; 2) conjunctival diseases; 3) history of ocular laser treatment, surgery, and trauma; 4) recent history of local ophthalmic medication; 5) history of ocular infections within the last 6mo; 6) recent wearers of contact lenses; 7) eyelid abnormalities; 8) patients diagnosed with rheumatic immune-related diseases, systemic immune system diseases, connective tissue diseases, or malignancies, such as Sjögren's syndrome, rheumatoid arthritis, Sicca syndrome, and lupus, *etc*.; 9) diseases or diagnoses affecting the central or peripheral nervous systems or other forms of neuropathy; 10) participants taking certain medications, such as antihistamines, tricyclic antidepressants, oral contraceptives, and antihypertensive drugs including diuretics were also excluded. Additionally, individuals with vitamin A deficiency and pregnant women were excluded; 11) individuals unable to cooperate in completing the examination.

Grouping Control Group: 20 cases without a history of diabetes; short duration group: diabetes duration ≤10y, 67 cases; long duration group: duration of diabetes >10y, 63 cases.

Research Methods All subjects were assessed by the Eye Table Disease Index Questionnaire (Ocular Surface Disease Index, OSDI). The severity of ocular surface symptoms was assessed according to the OSDI score grade proposed in literature: 0-12 normal, 13-22 mild, 23–32 moderate and 33-100 severe[9].

The examination methods for dry eye-related indicators have been standardized and will not be repeated in this article.

IVCM Examination Using the German Heidelberg laser confocal microscope (HRT-3, Hidelberg Engineering GmbH, Dossenheim, Germany) RCM module to observe the cornea and meibomian glands. The 10-30 clear images were selected for each eye from the center, upper, lower, nasal, and temporal sides, and ACCMetrics (University of Manchester, UK) fully automated analysis software was applied to measure the subepithelial nerves of the cornea, with all measurement results averaged. The nasal side, central, and temporal side of the lower eyelid meibomian gland near the eyelid margin were observed, 3 images from each area (a total of 9 images) were randomly selected, and ImageJ semi-automatic analysis software was applied to measure the meibomian gland-related indicators, with all measurement results averaged.

Statistical Analysis Data statistical analysis was performed using SPSS 27.0. The health control group and diabetes group measurements were tested for normality using the Shapiro-Wilk test. For indicators that were normally distributed, results are presented as mean±standard deviation (SD), the counting data were analyzed by Pearson Chi-square test; for those that were not normally distributed, results are presented as median and interquartile range [M (Q1, Q3)]. The Kruskal-Wallis *H* test was used for non-normally distributed data comparisons among multiple groups, analyzing the distribution between different groups, with pairwise comparison results adjusted using Bonferroni correction. Spearman rank correlation analysis was used to analyze the correlations between indicators. $P<0.05$ was considered statistically significant.

RESULTS

General Characteristics A total of 130 patients with type 2 diabetes were included in this study, among which 68 were male (52.3%) and 62 were female (47.7%), with an age range of 30 to 76y and an average age of (55.54±11.65)y. The control

group consisted of 20 cases, including 11 females (55.0%) and 9 males (45.0%), with an age range of 32 to 77y and an average age of (57.07±10.87)y.

Compared to the control group, there were no statistically significant differences in gender (χ^2=0.968, P=0.616) and age (H=5.226, P=0.073) among the three groups of subjects (Tables 1, 2).

OSDI Statistically significant differences in OSDI were found among the three groups (H=25.044, P<0.001). There was no significant difference between the control group and short-duration group (P=0.064), but there was a significant difference between the control and long-duration group and between the short-duration and long-duration group (P<0.001, P=0.001; Table 3).

BUT The differences of break-up time (BUT) between the three groups were statistically significant (H=23.962, P<0.001), and pairwise comparisons between the three groups showed statistically significant differences (P=0.007, P<0.001, P=0.015; Table 3).

SIT Significant differences in Schirmer I test (SIT) were observed among the three groups (H=24.704, P<0.001), and pairwise comparisons among the three groups also showed statistically significant differences (P=0.013, P<0.001, P=0.006; Table 3).

Corneal Nerve Fiber Density Using live-cell confocal microscopy, corneal nerve morphology was observed (Figure 1). Corneal nerves were analyzed using ACCMetrics, where red represents the main nerve trunk, blue represents branch nerves, and green represents nerve branching nodes.

The comparison of corneal nerve fiber density (CNFD) differences across the three groups were statistical significance (H=29.906, P<0.001), with the differences between the control group and the short disease duration, and between the control group and the long disease duration group, being statistically significant (P<0.001, P<0.001); however, there was no obvious statistical significance in the comparison between the short disease duration and long disease duration groups (P=0.070; Table 4).

Corneal Nerve Branch Density The comparison of corneal nerve branch density (CNBD) differences among the three groups was statistically significant (H=28.569, P<0.001). Specifically, the differences between the control group and the short and long disease duration groups were statistically significant (both P<0.001); however, there was no statistically significant difference between short and long disease duration groups (P=1.000; Table 4).

Corneal Nerve Fiber Length The differences in corneal nerve fiber length (CNFL) among the three groups were statistically significant (H=35.820, P<0.001), with significant differences between the control group and the short duration group (P<0.001) and between the control group and the long duration group (P<0.001); the difference between the short duration and long duration groups was statistically significant as well P=0.005 (Table 4).

Table 1 Gender distribution *n*

Groups	Female	Male	Total
Control group	11	9	20
Short duration DM	34	33	67
Long duration DM	28	35	63
Total	73	77	150

According to Pearson Chi-square test, χ^2=0.968, P=0.616. DM: Diabetes mellitus.

Table 2 Age distribution

Groups	Numbers	Age, y
Control group	20	[57.00 (54.0, 60.0)]
Short duration DM	67	[53.00 (47.5, 58.5)]
Long duration DM	63	[60.00 (53.5, 66.5)]

According to the Kruskal-Wallis H test, H=5.226, P=0.073. DM: Diabetes mellitus.

Table 3 Results of OSDI, TBUT, and SIT median (IQR)

Groups	OSDI	TBUT	SIT
Control group	4.0 (3.75, 5.25)	8.0 (5.0, 8.25)[c,d]	20.0 (17.0, 29.0)[f,g]
Short duration DM	8.0 (4.0, 12.0)	4.0 (3.0, 6.0)[e]	12.0 (9.0, 23.0)[h]
Long duration DM	12.0 (8.0, 16.0)[a,b]	3.0 (2.0, 5.0)	9.0 (5.0.0, 15.0)
H	25.044	23.962	24.704
P	<0.001	<0.001	<0.001

[a]P<0.001 *vs* control; [b]P=0.001 *vs* short duration DM; [c]P=0.007 *vs* short duration DM; [d]P<0.001 *vs* long duration DM; [e]P=0.015 *vs* long duration DM; [f]P=0.013 *vs* short duration DM; [g]P<0.001 *vs* long duration DM; [h]P=0.006 *vs* long duration DM. All comparisons were corrected using Bonferroni. OSDI: Ocular Surface Disease Index; TBUT: Tear break-up time; SIT: Schirmer I test; IQR: Interquartile range; DM: DM: Diabetes mellitus.

Diameters of Meibomian Gland Acini The morphology of meibomian gland acini was observed using IVCM (Figure 2).

The comparison of the longest diameter of the meibomian gland acini (MGALD) differences across the three groups were statistical significance (H=22.826, P<0.001), with statistically significant differences between the control group and both the short duration and long duration groups (P=0.001, P<0.001); however, the difference between the short duration and long duration groups was not statistically significant (P=0.144).

The comparison of the shortest diameter of meibomian gland acini (MGASD) differences across the three groups were statistical significance (H=23.188, P<0.001), with statistically significant differences between long duration group and both the control and the short duration groups (P=0.001, P<0.001); however, the difference between control group and the short duration group was not statistically significant (P=0.630; Table 5).

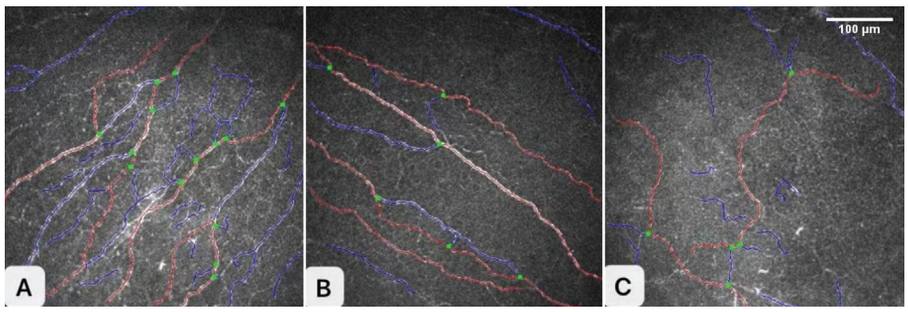

Figure 1 Corneal nerve morphology A: Control group; B: Short duration DM; C: Long duration DM. DM: Diabetes mellitus.

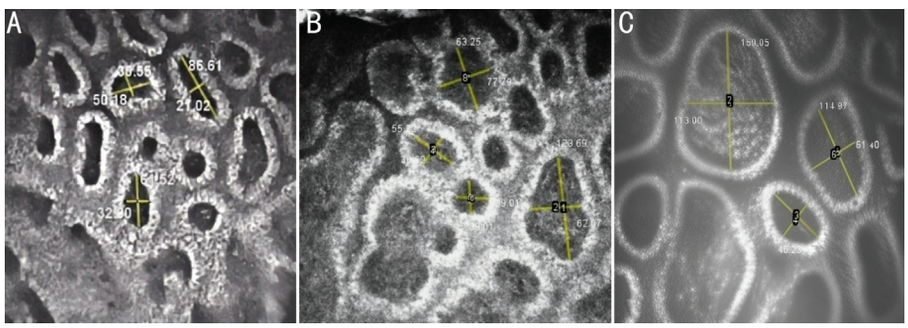

Figure 2 Acinar morphology of meibomian gland A: Control group; B: Short duration DM; C: Long duration DM. DM: Diabetes mellitus.

Table 4 Corneal nerve fiber related parameters median (IQR)

Groups	CNFD	CNBD	CNFL
Control group	25.42 (22.50, 27.66)[a,b]	46.36 (40.04, 48.28)[c,d]	18.17 (15.45, 20.40)[e,f]
Short duration DM	18.88 (16.32, 21.49)	29.68 (22.49, 33.44)	13.09 (11.69, 14.52)[g]
Long duration DM	16.90 (14.77, 19.66)	28.12 (21.87, 33.29)	11.55 (9.83, 13.55)
H	29.906	28.569	35.820
P	<0.001	<0.001	<0.001

[a]*P*<0.001 *vs* short duration DM; [b]*P*<0.001 *vs* long duration DM; [c]*P*<0.001 *vs* short duration DM; [d]*P*<0.001 *vs* long duration DM; [e]*P*<0.001 *vs* short duration DM; [f]*P*<0.001 *vs* long duration DM; [g]*P*=0.005 *vs* long duration DM, all comparisons use Bonferroni correction. CNFD: Corneal nerve fiber density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length; DM: Diabetes mellitus; IQR: Interquartile range.

Table 5 Meibomian gland-related parameters median (IQR)

Groups	MGALD	MGASD	MGAUD	MGAUA
Control group	99.28 (85.17, 107.93)[a,b]	15.15 (14.52, 17.25)[c]	117 (105, 129.75)[e]	1775.15 (1501.47, 1993.18)[f,g]
Short duration DM	114.32 (106.27, 123.31)	16.88 (14.15, 19.66)[d]	106 (98, 119.00)	2427.88 (1892.00, 3013.75)
Long duration DM	122.41 (108.32, 131.76)	20.13 (16.79, 16.87)	103 (97, 110.00)	2563.75 (2135.18, 2897.42)
H	22.826	23.188	8.856	16.188
P	<0.001	<0.001	<0.001	<0.001

[a]*P*=0.001 *vs* short duration DM; [b]*P*<0.001 *vs* long duration DM; [c]*P*=0.001 *vs* long duration DM; [d]*P*<0.001 *vs* long duration DM; [e]*P*=0.011 *vs* long duration DM; [f]*P*=0.002 *vs* short duration DM; [g]*P*<0.001 *vs* long duration DM, all comparisons were performed with Bonferroni correction. IQR: Interquartile range; MGALD: The longest diameter of the meibomian gland acini; MGASD: The shortest diameter of meibomian gland acini; MGAUD: Density of meibomian gland acinar units; MGAUA: The area of meibomian gland acinar units; DM: Diabetes mellitus.

Density and Area of Meibomian Gland Acini The comparison of density of meibomian gland acinar units (MGAUD) differences across the three groups were statistical significance (*H*=8.856, *P*=0.012), where there was no obvious statistical significance between the control group and the short duration group, and between the short duration group and the long duration group (*P*=0.137, *P*=0.375); however, there was a statistically significant difference between the control group and the long duration group (*P*=0.011; Table 5).

The comparison of the area of meibomian gland acinar units (MGAUA) differences among the three groups was statistically significant (*H*=16.188, *P*<0.001), with significant differences between the control group and the short disease duration and long disease duration (*P*=0.002, *P*<0.001); there was no statistical significance between the short disease duration and long disease duration (*P*=0.994; Table 5).

Correlation of Corneal Nerve and Meibomian Gland Indices with Disease Duration In the diabetes group, CNFD and CNFL were negatively correlated with the duration of diabetes (P<0.05); CNBD had no correlation with the duration of diabetes (P>0.05; Tables 6-7, Figures 3-5).

In the diabetes group, MGALD and MGASD were positively correlated with the duration of diabetes (P<0.05); whereas MGAUD and MGAUA were not correlated (P>0.05; Tables 8-9, Figures 6-9).

The relationship between gender, age, duration of diabetes, and ocular surface parameters in patients with type 2 diabetes was shown in Table 10. Specifically, the correlations between the duration of diabetes and the parameters related to corneal nerves were statistically significant. The duration of diabetes was significantly negatively correlated with CNFD, CNBD, and CNFL, indicating that as the duration of diabetes increased, corneal nerve damage occurred in diabetic patients; the density and length of corneal nerves decreased. The duration of diabetes was significantly positively correlated with MGALD and MGASD, indicating that as the duration of diabetes increases, the alveoli of the meibomian glands in diabetic patients partially dilate, with both the longest and shortest diameters increasing. However, no significant correlation was found with MGAUD and MGAUA, suggesting that the relationship between the duration of diabetes and MGAUD and MGAUA may be non-linear.

Table 6 Correlation between corneal nerve fibers and disease progression

Parameters	CNFD	CNBD	CNFL
r	-0.197	-0.139	-0.334
P	0.025	0.115	<0.001

CNFD: Corneal nerve fiber density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length.

Table 7 Quadratic fitting of the correlation between corneal nerve fibers and disease progression

Parameters	CNFD	CNBD	CNFL
R^2	0.103	0.015	0.137
Significance	0.001	0.387	<0.001

CNFD: Corneal nerve fiber density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length.

Table 8 Correlation between meibomian glands and disease duration

Parameters	MGALD	MGASD	MGAUD	MGAUA
r	0.180	0.401	-0.154	0.027
P	0.040	<0.001	0.080	0.764

MGALD: The longest diameter of the meibomian gland acini; MGASD: The shortest diameter of meibomian gland acini; MGAUD: Density of meibomian gland acinar units; MGAUA: The area of meibomian gland acinar units.

Table 9 Quadratic fitting of the correlation between meibomian glands and disease duration

Parameters	MGALD	MGASD	MGAUD	MGAUA
R^2	0.047	0.252	0.030	0.002
Significance	0.046	<0.001	0.144	0.857

MGALD: The longest diameter of the meibomian gland acini; MGASD: The shortest diameter of meibomian gland acini; MGAUD: Density of meibomian gland acinar units; MGAUA: The area of meibomian gland acinar units.

DISCUSSION

As the incidence of type 2 diabetes increases among common chronic diseases in our country, more and more people are paying attention to the ocular surface complications of type 2 diabetes, including decreased corneal sensitivity, MGD, dry eye, and conjunctival damage. This study focuses on the relevant effects of different disease durations on the corneal nerves and meibomian gland structures. We will analyze the similarities and differences by comparing our results with the conclusions of scholars both domestically and internationally.

In this study, there was a statistically significant difference in OSDI scores between the control group and different disease duration groups of diabetes, indicating that the discomfort of the ocular surface in patients with type 2 diabetes is more pronounced than in healthy individuals, making symptoms such as dry eye, stinging, foreign body sensation, and blurred vision more likely to occur. This is consistent with some previous studies[10-11]. The difference in OSDI scores among groups with different disease durations is statistically significant, suggesting that an extended duration of diabetes exacerbates ocular surface discomfort symptoms, which is consistent with the conclusion by Yu[12] that OSDI is significantly positively correlated with the duration of diabetes.

In this study, the difference in TBUT and SIT between the control group and the diabetic group was statistically significant, indicating that patients with type 2 diabetes have poorer tear film stability and tear secretion function compared to normal individuals. This is consistent with previous studies[13-14]. Because diabetes, as a systemic metabolic disease, is closely related to lipid metabolism, it can lead to the disruption of lipid homeostasis in the meibomian glands, subsequently affecting the synthesis of the lipid layer of the tear film, which is an important factor in maintaining tear film stability. Abnormalities in meibomian lipids or quantity can affect tear film stability[15].

As the duration of diabetes extends, the stability of the tear film shows a downward trend, and the secretion of tears gradually decreases. The reasons for this may be that the hyperglycemia environment activates the inflammation-related JNK-IKK-NFκB signaling pathway, leading to an increase in the expression of inflammatory factors; the hyperglycemia

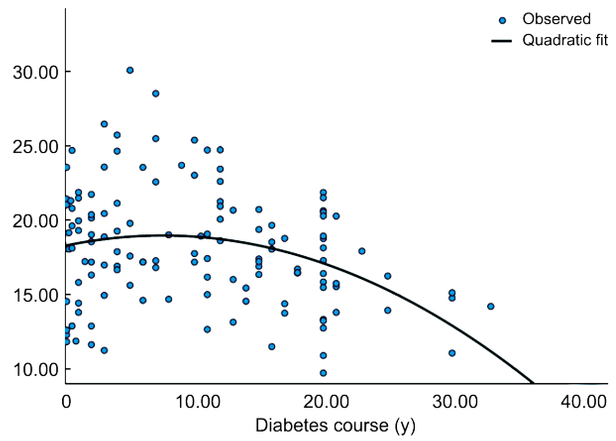

Figure 3 Scatter plot showing diabetes duration and corneal nerve fiber density (CNFD).

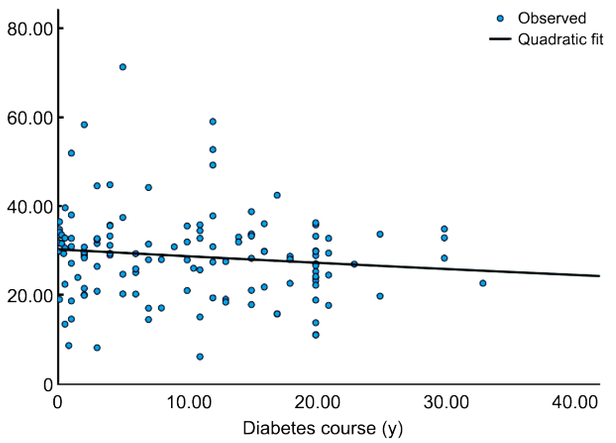

Figure 4 Scatter plot showing diabetes duration and corneal nerve branch density (CNBD).

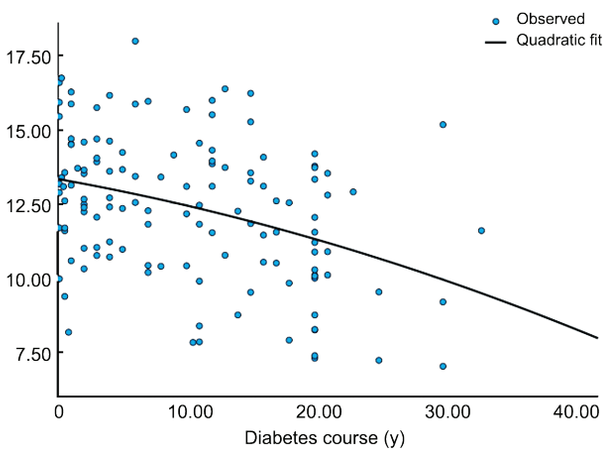

Figure 5 Scatter plot showing diabetes duration and corneal nerve fiber length (CNFL).

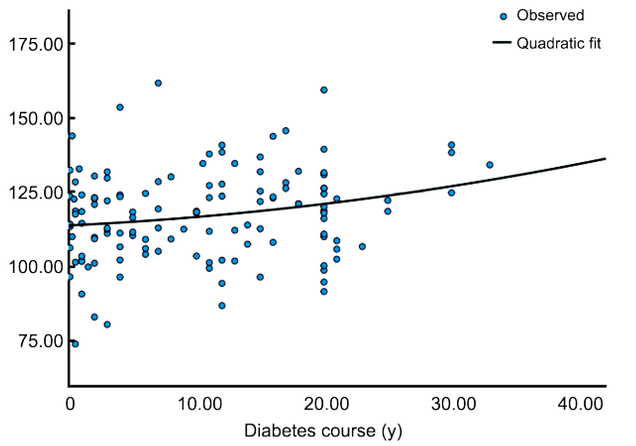

Figure 6 Scatter plot snowing diabetes duration and the longest diameter of the meibomian gland acini (MGALD).

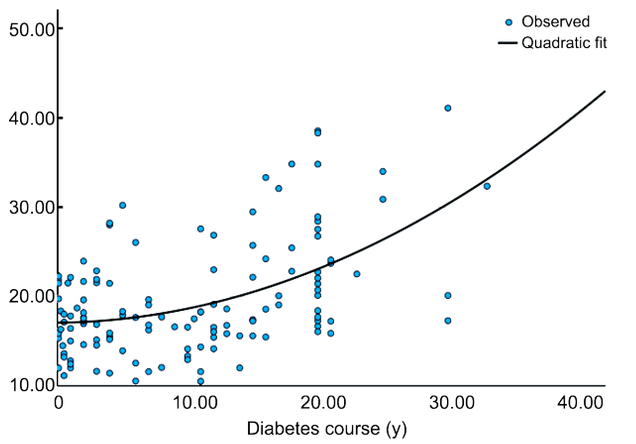

Figure 7 Scatter plot showing diabetes duration and the shortest diameter of meibomian gland acini (MGASD).

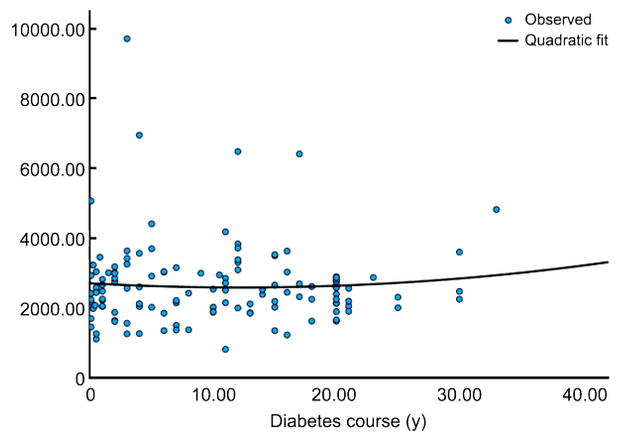

Figure 8 Scatter plot showing diabetes duration and the area of meibomian gland acinar units (MGAUA).

environment can also cause cell osmotic edema through the polyol pathway, while the production and accumulation of advanced glycation end products affect cell proliferation and differentiation, resulting in damage to the lacrimal glands and cornea, ultimately leading to reduced tear secretion and poor tear film stability[16-17].

Corneal nerves radially penetrate the cornea *via* the stroma, undergo bifurcation, and extend toward the epithelium, presenting as long nerve bundles, delicate branches, and nerve terminals. In this study, the CNFD, CNBD, and CNFL of the diabetic group significantly decreased compared to the healthy control group, indicating that patients with type 2 diabetes developed corneal nerve damage, with reduced corneal nerve density and length, consistent with previous studies[18-20]. Research[21] pointed out that the decrease in CNFD, CNBD, and CNFL is symmetrical in both eyes and closely related to

Table 10 Multiple linear regression analysis: standardized coefficients for factors associated with ocular surface parameters β (P)

Independent variable	CNFD	CNBD	CNFL	MGALD	MGASD	MGAUD	MGAUA
Gender	-0.623 (0.439)	-4.254 (0.119)	0.089 (0.886)	-2.191 (0.391)	-0.035 (0.972)	4.563 (0.138)	4.702 (0.982)
Age, y	-0.145 (0.037)	-0.004 (0.982)	0.002 (0.957)	-0.183 (0.151)	-0.021 (0.684)	-0.164 (0.300)	5.215 (0.634)
Disease duration, y	-0.313 (<0.001)	-0.507 (0.005)	-0.175 (<0.001)	0.403 (0.005)	0.381 (<0.001)	-0.268 (0.146)	-0.477 (0.970)

CNFD: Corneal nerve fiber density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length; MGALD: The longest diameter of the meibomian gland acini; MGASD: The shortest diameter of meibomian gland acini; MGAUD: Density of meibomian gland acinar units; MGAUA: The area of meibomian gland acinar units.

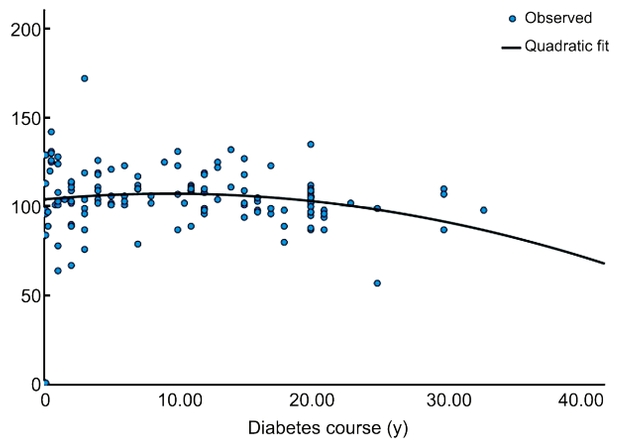

Figure 9 Scatter plot showing diabetes duration and density of meibomian gland acinar units (MGAUD).

the duration of diabetes. Although there was no statistically significant difference in CNFD and CNBD between the short-duration and long-duration groups in this study, Spearman correlation analysis showed a negative correlation between CNFD, CNFL, and duration, while CNBD showed no correlation with duration. This suggests that with the gradual extension of duration, there is a decreasing trend in CNFD and CNFL, but the change in the number of CNBD is not significant, indicating that the main corneal trunk nerves may undergo atrophy, leading to a general decrease in nerve density, and the extent of corneal nerve damage is worsening. This is consistent with previous studies[22-24].

The mechanisms that may contribute to this change include: sustained hyperglycemia leading to the accumulation of advanced glycation end products, enhanced activity of the polyol pathway, increased oxidase, and the protease K pathway resulting in demyelination of neurons and impairment of corneal nerve innervation[25]. Research[26] has confirmed that poly (ADP-ribose) polymerase may trigger oxidative stress mechanisms, resulting in increased production of reactive oxygen species, thereby causing mitochondrial damage. Yagihashi *et al*[27] indicated that mitochondrial damage in nerve fibers may lead to demyelination and conduction dysfunction. Additionally, literature points out that mechanisms leading to decreased corneal nerve density may include changes in extracellular matrix components such as collagen, proteoglycans, and fibronectin in the corneal stroma, which are upregulated in diabetes and may disrupt the process of nerve growth[28-29]; there may also be changes in basement membrane thickness and molecular composition, increasing the resistance of stromal nerve branches to penetrate the epithelium, resulting in decreased corneal nerve density and length in diabetic patients[30]. Research indicates that the levels of insulin-like growth factor binding protein-3 in the tears of diabetic patients are elevated 3.5 times and are highly correlated with corneal nerve fiber length and branching density, showing a significant correlation with corneal nerve loss[18].

Among the morphologically related parameters of meibomian gland acini, MGALD and MGASD reflect the overall size of the acini, MGAUD reflects the density of acini within a unit area, and MGAUA reflects the average area per acinus within a unit area. In this study, there were statistically significant differences in MGAUD, MGALD, MGASD, and MGAUA between the healthy control group and groups with varying durations of diabetes. In diabetic patients, MGALD and MGASD are higher than normal, MGAUD is lower than normal, and MGAUA is higher than normal. In the linear correlation analysis, the duration of diabetes was significantly positively correlated with MGALD and MGASD. Moreover, after quadratic fitting, it was indicated that with increasing diabetes duration, the alveoli of the meibomian glands in diabetic patients partially dilated, and both the longest and shortest diameters increased. There was no significant correlation between diabetes duration and MGAUD and MGAUA, and the correlation remained insignificant after quadratic fitting. This indicates that there is a non-linear correlation and no curve correlation between diabetes duration and MGAUD and MGAUA. However, Spearman rank correlation analysis showed that MGAUA was positively correlated with the duration of the disease. MGAUD is negatively correlated with the duration of the disease, indicating that as the duration of diabetes increases, MGAUA shows a monotonically increasing trend and MGAUD shows a monotonically decreasing trend, but the pattern of change is irregular. The reason for this relationship may be due to the different proportions of atrophy and dilation of meibomian

gland follicles in patients. Overall, it can be understood that as the disease progresses, the overall volume of the acinar glands shows an increasing trend, while the density shows a decreasing trend. This is inconsistent with the results of previous studies[19-20]. The study[12] suggests that MGASD increases with the prolongation of the disease duration, MGALD shows no significant change with the prolongation of the disease duration, MGAUD gradually decreases with the prolongation of the disease duration, and MGAUA varies with the disease duration.

The analysis of the structural morphological effects of diabetes on the meibomian glands may have several reasons: first, a high glucose environment induces a large number of inflammatory cytokines, and oxidative stress leads to cellular tissue damage; second, hyperkeratosis of the meibomian gland cells causes duct blockage; third, diabetes affects systemic lipid metabolism, resulting in abnormal lipid secretion from the meibomian glands; fourth, the abnormal hormone levels in diabetic patients, especially the decrease in estrogen levels, exacerbate apoptosis of the meibomian gland epithelial cells, leading to gland atrophy and loss[20]. Patients with MGD due to diabetes may experience glandular acinar dilation or atrophy, with acinar dilation being particularly evident near the eyelid margin and a notable increase in acinar diameter, while acini near the dome area mostly present in a state of atrophy with significantly reduced acinar diameter[20].

In summary, we can see that patients with type 2 diabetes are more prone to experience damage to the corneal nerves and meibomian glands, with symptoms of ocular surface discomfort being more pronounced than in healthy individuals, and worsening as the duration of diabetes increases. Changes in the morphology and structure of the cornea and meibomian glands become more evident over time. This study is the first to use IVCM to simultaneously observe the morphological changes of corneal nerves and meibomian glands in patients with type 2 diabetes, analyze their correlation with the duration of diabetes, and explore the mechanism behind the structural abnormalities on the ocular surface in type 2 diabetes patients, which aids in the diagnosis of diabetic ocular surface diseases and facilitates the early detection of diabetic peripheral neuropathy. In addition, IVCM, characterized by its non-invasive and high-resolution nature, allows for real-time, dynamic observation of the ultrastructural changes of corneal nerves and meibomian glands, yielding more refined results compared to traditional methods such as slit-lamp examination and LipiView. In contrast to the evaluation of intraepidermal nerve fiber density through skin biopsy, the same corneal region can be analyzed repeatedly, which is valuable for longitudinal investigations to monitor disease progression and evaluate therapeutic efficacy[31]. Corneal confocal microscopy can differentiate painful diabetic peripheral neuropathy (20%-30% of cases) from painless diabetic peripheral neuropathy by detecting more severe nerve loss, thus facilitating clinical phenotype stratification[32]. Future advancements in artificial intelligence integration and wider accessibility may contribute to better prognosis for neurological and ophthalmological disorders.

ACKNOWLEDGEMENTS

This thesis is the graduation thesis of a master's student (Han YZ).

Authors' Contributions: Zhang Y, Han YZ, Zhao ZH, Fan F, Chen YM, and Wang JM examined all the subjects and wrote the paper; Li KJ has critically reviewed and revised the paper.

Foundations: Supported by 2019 Medical Science Research Key Project Plan of Hebei Province (No.201900341); 2024 Medical Science Research Key Project Plan of Hebei Province (No.20242157); the Natual Science Foundation of Hebei Province (No.H2020206650); Hebei Provincial Government Funded Clinical Medicine Excellent Talents Project; Hebei Province Medical Application Technology Tracking Project (No.GZ2023091).

Conflicts of Interest: Li KJ, None; **Zhang Y,** None; **Han YZ,** None; **Zhao ZH,** None; **Fan F,** None; **Chen YM,** None; **Wang JM,** None.

REFERENCES

1 Zhang R, Wang MY, Zhang XQ, *et al*. Self-care activities mediate self-perceived burden and depression in Chinese patients with type 2 diabetes. *World J Psychiatry* 2025;15(5):104766.

2 Santos GL, dos Santos CF, Rocha GR, *et al*. Beyond glycemic control: Roles for sodium-glucose cotransporter 2 inhibitors and glucagon-like peptide-1 receptor agonists in diabetic kidney disease. *World J Diabetes* 2025;16(6):104706.

3 Ljubimov AV. Diabetic complications in the cornea. *Vis Res* 2017;139:138-152.

4 Wu HP, Fang X, Luo SR, *et al*. Meibomian glands and tear film findings in type 2 diabetic patients: a cross-sectional study. *Front Med* 2022;9:762493.

5 Yu FX, Lee PSY, Yang LL, *et al*. The impact of sensory neuropathy and inflammation on epithelial wound healing in diabetic corneas. *Prog Retin Eye Res* 2022;89:101039.

6 Singh I, Poynten AM, Krishnan AV, *et al*. The ocular surface in type 2 diabetes: pathophysiology and impact of anti-diabetic drugs. *Prog Retin Eye Res* 2026;110:101417.

7 Huang ES, Laiteerapong N, Liu JY, *et al*. Rates of complications and mortality in older patients with diabetes mellitus: the diabetes and aging study. *JAMA Intern Med* 2014;174(2):251-258.

8 Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. *Eye (Lond)* 1991;5(4):395-411.

9 Miller KL, Walt JG, Mink DR, et al. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol* 2010;128(1):94-101.

10 Hao YR, Wu BG, Feng J, *et al*. Relationship between type 2 diabetes mellitus and changes of the lid margin, meibomian gland and tear film in dry eye patients: a cross-sectional study. *Int Ophthalmol* 2025;45(1):261.

11 Manchikanti V, Kasturi N, Rajappa M, *et al*. Ocular surface disorder among adult patients with type II diabetes mellitus and its correlation with tear film markers: a pilot study. *Taiwan J Ophthalmol* 2021;11(2):156-160.

12 Yu T. Morphological and cytological changes of meibomian glands in patients with type 2 diabetes mellitus. *Int J Ophthalmol* 2019;12(9):1415-1419.

13 Li KJ, Han YZ, Zhang Y, *et al*. Analysis of the correlation between corneal and meibomian gland morphology and glycated hemoglobin levels in type 2 diabetes patients. *BMC Ophthalmol* 2025;25(1):480.

14 Silva-Viguera MC, Pérez-Barea A, Bautista-Llamas MJ. Tear film layers and meibomian gland assessment in patients with type 1 diabetes mellitus using a noninvasive ocular surface analyzer: a cross-sectional case–control study. *Graefes Arch Clin Exp Ophthalmol* 2023;261(5):1483-1492.

15 Bai YQ, Ngo W, Khanal S, *et al*. Human precorneal tear film and lipid layer dynamics in meibomian gland dysfunction. *Ocul Surf* 2021;21:250-256.

16 Alves M, Calegari VC, Cunha DA, *et al*. Increased expression of advanced glycation end-products and their receptor, and activation of nuclear factor kappa-B in lacrimal glands of diabetic rats. *Diabetologia* 2005;48(12):2675-2681.

17 Fasanella V, Agnifili L, Mastropasqua R, *et al*. In vivo laser scanning confocal microscopy of human meibomian glands in aging and ocular surface diseases. *BioMed Res Int* 2016;2016:7432131.

18 Stuard WL, Titone R, Robertson DM. Tear levels of insulin-like growth factor binding protein 3 correlate with subbasal nerve plexus changes in patients with type 2 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2017;58(14):6105-6112.

19 Swiderska K, Blackie CA, Maldonado-Codina C, *et al*. Evaluation of Meibomian gland structure and appearance after therapeutic Meibomian gland expression. *Clin Exp Optom* 2024;107(5):504-514.

20 Gao C. In vivo confocal microscopy evaluation of meibomian glands in meibomian gland dysfunction patients. *Shanxi Medical University* 2017.

21 Petropoulos IN, Alam U, Fadavi H, *et al*. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. *Diabetes Care* 2013;36(11):3646-3651.

22 Fang W, Lin ZX, Yang HQ, *et al*. Changes in corneal nerve morphology and function in patients with dry eyes having type 2 diabetes. *World J Clin Cases* 2022;10(10):3014-3026.

23 Maddaloni E, Sabatino F, Del Toro R, *et al*. *In vivo* corneal confocal microscopy as a novel non-invasive tool to investigate cardiac autonomic neuropathy in Type 1 diabetes. *Diabet Med* 2015;32(2):262-266.

24 Liu SY, Yang B, Zhou XH, *et al*. Correlation of glycosylated hemoglobin and superoxide dismutase levels with corneal nerve injury in type 2 diabetes mellitus. *Henan Medical Research* 2022;31(11):2012-2015.

25 Bu YS, Shih KC, Tong L. The ocular surface and diabetes, the other 21st Century epidemic. *Exp Eye Res* 2022;220:109099.

26 Zhao H. Corneal alteration and pathogenesis in diabetes mellitus. *Int J Ophthalmol* 2019;12(12):1939-1950.

27 Yagihashi S, Mizukami H, Sugimoto K. Mechanism of diabetic neuropathy: Where are we now and where to go? *J Diabetes Invest* 2011;2(1):18-32.

28 He JC, Bazan HEP. Mapping the nerve architecture of diabetic human corneas. *Ophthalmology* 2012;119(5):956-964.

29 He JC, Bazan HEP. Epidermal growth factor synergism with TGF-β1 *via* PI-3 kinase activity in corneal keratocyte differentiation. *Invest Ophthalmol Vis Sci* 2008;49(7):2936-2945.

30 Radwan SE, El-Kamel AH, Zaki EI, *et al*. Hyaluronic-coated albumin nanoparticles for the non-invasive delivery of apatinib in diabetic retinopathy. *Int J Nanomed* 2021;16:4481-4494.

31 Lukashenko MV, Gavrilova NY, Bregovskaya AV, *et al*. Corneal confocal microscopy in the diagnosis of small fiber neuropathy: faster, easier, and more efficient than skin biopsy. *Pathophysiology* 2021;29(1):1-8.

32 Kalteniece A, Ferdousi M, Azmi S, *et al*. Corneal confocal microscopy detects small nerve fibre damage in patients with painful diabetic neuropathy. *Sci Rep* 2020;10(1):3371.