

BMJ Open Relationship between ocular surface pain and corneal nerve loss in dry eye diabetics: a cross-sectional study in Shenyang, China

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ABSTRACT

Background Diabetes mellitus has been associated with increased dry eye disease (DED) and exacerbates DED pathology.

Objective To investigate the potential relationship between corneal nerve loss and ocular pain among diabetic patients with dry eye (DE).

Design A cross-sectional study.

Setting He Eye Specialist Hospital, Shenyang, China.

Participants This study recruited 124 eyes of 62 diabetic patients diagnosed with DED between August and October 2022.

Main outcome measures Best-corrected visual acuity, intraocular pressure, non-invasive tear breakup time, tear meniscus height, tear film lipid layer, conjunctival hyperaemia (redness score), conjunctivocorneal epithelial staining (CS score), central corneal sensitivity and *in vitro* confocal corneal microscopy was assessed in all subjects. The Ocular Surface Disease Index Questionnaire assessed DE symptoms and ocular pain.

Results The study's final analysis included 26 patients (52 eyes) without ocular pain and 36 patients (72 eyes) with ocular pain. The corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fibre length (CNFL) in patients with ocular pain were significantly lower than those without ($p < 0.001$, $p = 0.004$, and $p < 0.001$, respectively). CNFD, CNBD and CNFL negatively correlated with ocular pain ($r = -0.385$, $r = -0.260$, $r = -0.358$, respectively). Moreover, CNFD, CNBD and CNFL have a significant ($p < 0.05$) positive correlation with corneal sensitivity ($r = 0.523$, $r = 0.330$, $r = 0.421$, respectively).

Conclusions Corneal nerve loss was associated with ocular pain and decreased corneal sensitivity in diabetic patients with DE. Further studies into the neurological role of ocular surface diseases can elaborate diagnostics, prognosis and treatment of diabetic patients with DE.

Trial registration number ClinicalTrials.gov (NCT05193331).

INTRODUCTION

A loss of tear film homeostasis characterises dry eye disease (DED). It is accompanied by ocular symptoms, tear film instability,

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ A comprehensive evaluation of tear film, corneal sensitivity and dry eye symptom were performed.
- ⇒ *In vitro*, confocal corneal microscopy examined corneal nerve alterations in diabetic patients with dry eye.
- ⇒ This study did not include healthy participants or dry eye patients without diabetes as a comparative group.
- ⇒ The possible effects of blood glucose levels are unclear due to the lack of blood glucose testing.

hyperosmolarity, damage and neurosensory abnormalities.¹ Diabetes mellitus (DM) is one of the risk factors for DED; 47% of patients with DM suffer from ocular surface damage due to negative alterations to the tear film, corneal thickness, corneal epithelium, corneal nerve and corneal endothelium.^{2–4} The prevailing consensus implies that DM affects the microvasculature of the lacrimal gland and corneal nerve sensation. These changes thereby affect the homeostasis of the tear film, resulting in ocular surface insult and, consequently, exacerbating the signs and symptoms of DED.^{5,6}

DED can have various symptoms, including irritation, blurred vision and ocular pain.⁷ Ocular pain is more common in diabetic patients with dry eye (DE) due to corneal nerve damage caused by the dual effects of systemic high glucose environment and tear film instability.⁸ The cornea is primarily innervated by the ophthalmic branch of the trigeminal nerves (cranial nerve V), crucial physiological system elements that regulate ocular surface homeostasis.^{9,10} A previous study reported a loss of 6% or more of corneal nerve fibres per year in 17% of patients with diabetes.¹¹ The sensory-discriminative components of ocular

pain and maintaining ocular homeostasis appear to be significantly influenced by these neurons.^{7 12}

Corneal nerves govern blinking, tear production, pain, warmth and touch sensations.¹³ Additionally, corneal nerves generate neurotrophic substances that preserve ocular tissue homeostasis and function.¹⁴ Corneal Langerhans cells, or corneal dendritic cells, are inflammatory, antigen-presenting corneal cells that maintain corneal homeostasis and immune surveillance.¹⁵ The basal epithelial layer to the sub-basal nerve plexus (SNP) includes these cells. Recent research indicates that corneal nerve loss is related to the severity of painful diabetic neuropathy (PDN), the former having predictive value for the PDN.^{12 16} Additionally, a prior study reported corneal nerve width was positively connected with the Ocular Surface Disease Index (OSDI) Score, blurred vision and painful eyes in the general population.¹⁷ However, the relationship between corneal nerve damage and ocular pain in diabetic patients with DE has not been explored. This study investigated the potential connection between corneal nerve loss and ocular pain among diabetic patients with DE.

METHODS

Study design and participants

This cross-sectional study recruited 124 eyes of 61 diabetic patients diagnosed with DED between August and October 2022. The study protocol was approved by the Ethics Committee of He Eye Specialist Hospital (IRB (2022) K002.01) and adhered to the tenets of the Declaration of Helsinki. The study was registered with ClinicalTrials.gov (NCT05193331). All subjects signed informed consent forms before they participated in the study.

Inclusion criteria: patients previously diagnosed with diabetes were informed and enrolled in this study. (1) Age ≥ 18 years, (2) clinically diagnosed and confirmed with DM for 1 year or more, (3) able and willing to adhere to the therapy and follow-up plan, (4) participants were diagnosed with DE according to the Tear Film and Ocular Surface Society's Dry Eye Workshop II (TFOS DEWS II) diagnostic criteria: (a) OSDI Questionnaire ≥ 13 , (b) non-invasive tear breakup time (NITBUT) < 10 s, (c) ocular surface staining > 5 corneal spots, greater than nine conjunctival spots (the presence of two or more criteria was used to establish a positive DE diagnosis).

Exclusion criteria: (1) active ocular infection, such as infectious, viral, chlamydial or immunological conjunctivitis; (2) a history of ocular surgery that might affect the corneal nerve, such as corneal refractive surgery, keratoplasty or ocular laser surgery; (3) long-term contact lens wear; and (4) other diseases that may cause ocular pain, such as glaucoma and trigeminal neuralgia.

Patient and public involvement

All subjects signed informed consent forms before they participated in the study.

Clinical evaluations

The following tests were given to all subjects, including best-corrected visual acuity, intraocular pressure, NITBUT, tear meniscus height (TMH), tear film lipid layer (TFLL), conjunctival hyperaemia (redness score (RS)), conjunctivocorneal epithelial staining (CS score), central corneal sensitivity, and *in vitro* confocal corneal microscopy.

The DE diagnostic system assessed NITBUT and TMH (MediWorks, Shanghai, China). Three consecutive measurements were taken, and the median value was entered as the final reading.

TFLL score interferometry was evaluated using DR-1 instrument (Kowa, Nagoya, Japan). According to Hosaka *et al.*¹⁸ grading method, TFLL quality was categorised, and a lower number indicates higher TFLL quality.

RS was assessed by Keratography 5M (Oculus, Germany), and the score can range from 0.0 (normal) to 4.0 (severe).

CS score measures corneal and conjunctival epithelium damage using the methods proposed by Arita *et al.*¹⁹ A preservative-free solution containing 1% lissamine green and 1% sodium fluorescein was instilled in the conjunctival sac with 2 mL of a double vital staining approach. As formulated by The Asia Dry Eye Society,²⁰ the ocular surface was sectionalised into three equal sections representing the temporal conjunctiva, cornea and nasal conjunctiva. Each region was given a maximum staining score of 3 points and a minimum staining score of 0. The combined scores from all three parts were then recorded on a scale ranging from 0 (normal) to 9 (severe).

Central corneal sensitivity was measured using a Cochet-Bonnet esthesiometer (Luneau Technology Operations, France), which stimulates the cornea with a nylon monofilament. The stiffness of the filament is adjusted by altering the length (0–6 cm) of the filament with a slider on the side of the pen.²¹

The *in vitro* confocal corneal microscopy images were obtained by HRT III RCM (Heidelberg Engineering GmbH, Dossenheim, Germany), which was used to examine corneal nerve alterations. For each eye, three central corneal SNP images that were non-overlapping, high-contrast and high-quality were chosen for analysis. The measurement of corneal nerve morphology was carried out using fully automated analysis software (ACCMetrics).²² The total number of main nerves per square millimetre: corneal nerve fibre density (CNFD) (no./mm^2); the total number of branches per square millimetre: corneal nerve branch density (CNBD) (no./mm^2); and the total length of main nerves and nerve branches per square millimetre: corneal nerve fibre length (CNFL) (mm/mm^2) were quantified.

Assessment of symptoms

A validated Chinese version of OSDI provided a quantifiable assessment of DE symptoms and ocular pain.²³ The 12 items of the questionnaire can be tabulated to obtain an individual score ranging from 0 to 100 (no symptoms to severe symptoms) points. Patients answered two OSDI

Table 1 Demographics comparison

	No pain	Pain	P value
No. of eyes	52	72	
Sex, male, n (%)	17 (32.7 %)	21 (29.2%)	0.674
Age (years)	59.73±11.58	60.69±12.00	0.655
BCVA (LogMAR)	0.10±0.15	0.15±0.18	0.135
IOP (mm Hg)	16.35±2.67	16.37±2.35	0.968
BCVA, best-corrected visual acuity; FBG, fasting blood glucose; IOP, intraocular pressure.			

Questionnaires based on left and right ocular symptoms. According to item 3 of OSDI, 'Painful or sore eyes' (0–4 score), patients were categorised into groups without and with ocular pain.

Statistical analyses

The statistical analyses were conducted using SPSS statistics software (V.25.0; SPSS, USA). Mean standard deviation ($\pm$ SD) was used to express descriptive statistics for continuous variables, whereas number (percentage) was used for binary variables. The χ^2 test was used for categorical data for analysis. Using the Kolmogorov-Smirnov test, the normality of the variables was determined. The means of all ocular parameters for individuals with diabetes with ocular pain and no pain were compared. The Student's t-test was used to compare parameters with a normal distribution, while the Mann-Whitney U test was employed for non-normally distributed values. Spearman's rho correlation was used to establish the relationships between corneal nerve and variables. A p value ≤ 0.05 was considered as statistical significance.

RESULTS

Demographic and ocular surface parameters

The final analysis included 26 patients (52 eyes) without ocular pain and 36 patients (72 eyes) with ocular pain. Patients with and without ocular pain were matched for age ($p=0.655$) and gender ($p=0.674$) (table 1).

The ocular surface parameters are presented in table 2. Corneal sensitivity in patients with ocular pain was significantly lower than in patients without ocular pain ($p<0.001$) (figure 1). The OSDI Score in patients with ocular pain was considerably higher than those without ocular pain ($p<0.001$). The CNFD, CNBD and CNFL in patients with ocular pain were substantially lower than those without ($p<0.001$, $p=0.004$ and $p<0.001$, respectively). There was no significant difference in NITBUT ($p=0.903$), TMH ($p=0.488$), TFL ($p=0.502$), CS score ($p=0.150$) and RS ($p=0.964$) between groups of patients.

Correlation between ocular surface parameters

The correlation between corneal nerve fibre and ocular surface parameters in patients with diabetes and DE is shown in table 3. In patients with diabetes and DE, CNFD, CNBD and CNFL negatively correlated with ocular pain

Table 2 Comparison of ocular surface parameters

	No pain	Pain	P value
NITBUT (sec)	6.45±4.70	5.41±2.57	0.903
CS score (0–9)	1.37±1.24	1.13±1.44	0.150
TFL (1–5)	2.79±0.57	1.71±0.46	0.502
Corneal sensitivity (1–6)	5.71±0.72	5.04±1.27	<0.001
TMH (mm)	0.20±0.08	0.21±0.10	0.488
RS (0–4)	1.54±0.43	1.54±0.51	0.964
OSDI (0–100)	26.99±14.77	43.07±18.71	<0.001
CNFD (no./mm ²)	23.32±5.27	18.14±6.73	<0.001
CNBD (no./mm ²)	32.50±15.40	24.03±13.12	0.004
CNFL (mm / mm ²)	14.50±3.52	11.63±3.54	<0.001
CNBD, corneal nerve branch density; CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CS, corneal conjunctival staining; NITBUT, non-invasive tear break-up time; OSDI, Ocular Surface Disease Index; RS, redness score; TFL, tear film lipid layer; TMH, tear meniscus height.			

(figure 2) and OSDI. The correlation analysis between the corneal nerve fibre and other ocular surface parameters, including NITBUT, TMH, TFL, CS score and RS, showed no statistical significance. However, CNFD, CNBD and CNFL have a positive correlation with corneal sensitivity.

DISCUSSION

DM is a developing global health challenge due to the multiple complications associated with long-term hyperglycaemia.²⁴ Although diabetic retinopathy is the most prevalent and well-known ophthalmic consequence, diabetes also causes clinically significant effects on the ocular surface.^{25 26} It has been documented that patients with diabetes have a higher prevalence of DE than healthy individuals.²⁷ Confocal microscopy is an emerging non-invasive technique that has advanced our understanding

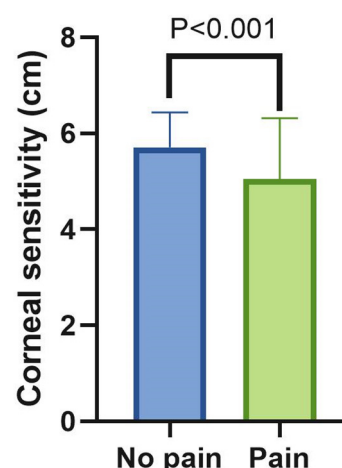
**Figure 1** Comparison of corneal sensitivity between groups.

Table 3 Correlation between ocular surface parameters and corneal nerve morphology

	Ocular pain	NITBUT	TMH	TFLL	Corneal sensitivity	CS	RS	OSDI
CNFD	0.385**	NS	NS	NS	0.523**	NS	NS	0.196*
CNBD	0.260**	NS	NS	NS	0.330**	NS	NS	0.177*
CNFL	0.358**	NS	NS	NS	0.421**	NS	NS	0.309**

NS: correlation was detected during correlation analysis.

Significant correlation: * $p < 0.05$; ** $p < 0.01$.

CNBD (no./mm²), corneal nerve branch density; CNFD (no./mm²), corneal nerve fibre density; CNFL (mm/mm²), corneal nerve fibre length; CS, corneoconjunctival staining; NITBUT, non-invasive tear break-up time; OSDI, Ocular Surface Disease Index; RS, redness score; TFLL, tear film lipid layer; TMH, tear meniscus height.

of ocular surface diseases on a microscopic level.²⁸ In the current study, corneal nerve fibre was assessed by confocal microscopy among diabetic patients with DE, and the findings indicate that corneal nerve loss was associated with ocular pain and decreased corneal sensitivity.

DED may result from various factors such as dysfunction with the meibomian glands, the tear-secreting glands or the neural circuits controlling these glands.²⁷ Regardless, reduced corneal sensitivity and neurosensory abnormalities are now understood as standard features.¹ Disturbance to corneal innervation has been implicated in diabetic patients with DED.⁹ Our investigation showed that decreasing corneal sensitivity was related to more significant corneal nerve loss. Moreover, corneal confocal microscopy revealed increasing ocular pain and corneal nerve fibre loss among diabetic patients with DE. According to Galor *et al*, DE disease is a somatosensory dysfunction.²⁹ Damage to sensory nerve endings may be accompanied by sensitisation to ongoing electrochemical activity in injured nerve fibres, trigeminal neurons and higher-order neurons, leading to neuropathic pain

symptoms,⁷ resulting in exacerbated signs and symptoms of DE. Similarly, Liu and colleagues also reported that the severity of a variety of DE symptoms might be related to corneal nerve width and tortuosity in the normal population.¹⁷ In contrast to Han *et al*,³⁰ we found no relationships between corneal nerve fibre loss and objective indices of DE, possibly due to the lack of diabetes duration-matched comparative group.

Labetoulle *et al* reported that neural pathology plays a crucial and independent role in DED beyond tear dysfunction.⁹ While artificial tears can partially alleviate the ocular pain experienced by DE patients, it is suggested that these individuals would benefit from multimodal therapy that also considers corneal innervation.^{31 32} The study highlights that there were significantly fewer corneal nerve fibres in patients with ocular pain than in those without ocular pain, suggesting that in clinical practice, corneal confocal microscopy should be considered for diabetic patients with DE and ocular pain. These individuals would benefit from multimodal therapy, which includes possible neuroprotective agents,

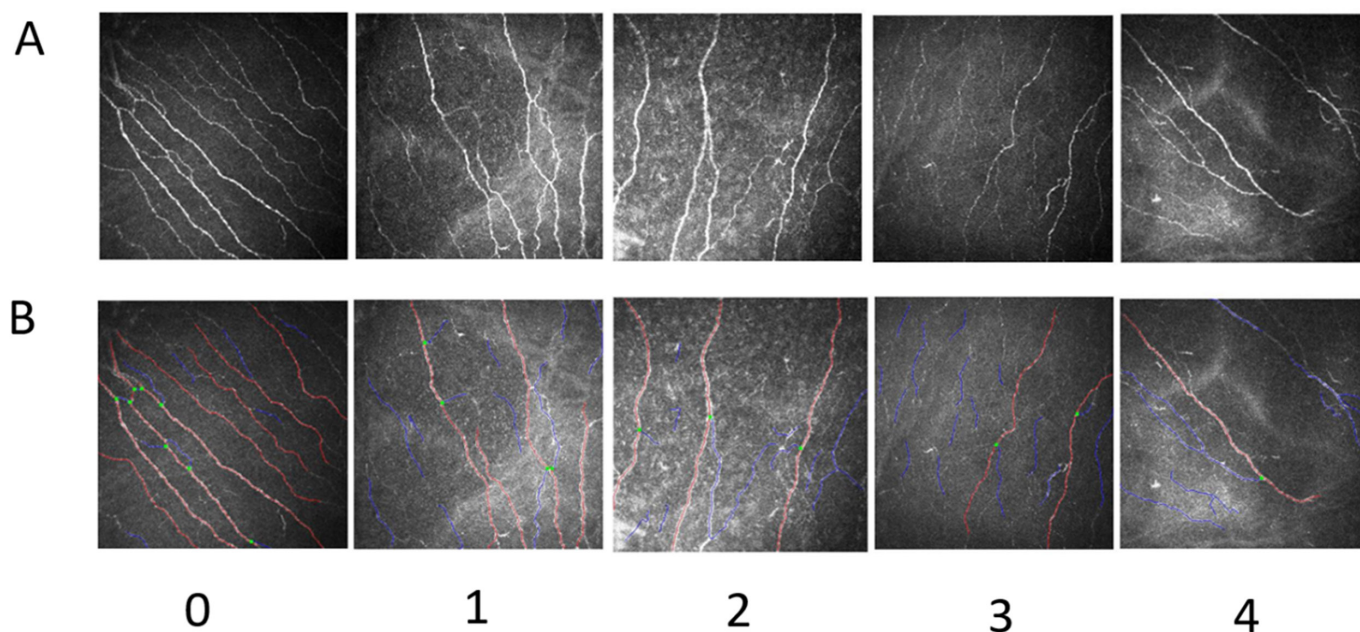


Figure 2 Corneal sub-basal nerve plexus analysis. (A) The original image of corneal sub-basal nerve plexus morphology. (B) Nerves detected with the fully automated analysis software (ACCMetrics). 0–4: the score of ‘Painful or sore eyes’ (never to constantly), as derived by Kalteniece *et al*.¹⁶

including ciclosporin, nerve growth factor, docosahexaenoic acid, topical citicoline and vitamin B₁₂.^{33–37} Especially topical insulin is indispensable as first-line therapy and proper glycaemic control is essential to avoid diabetes complications.¹²

The limitation of this study is that it does not include healthy participants or DE patients without diabetes as comparative groups. Furthermore, the possible effects of blood glucose levels are unclear due to the lack of blood glucose test reports. However, the focus of the study was to explore the correlation of corneal nerves with ocular pain symptoms to further explain the role of ocular surface neural changes among diabetic patients with DE. Future research will aim to stratify DE, pain severity, and severity of peripheral neuropathy.

In conclusion, in vitro confocal corneal microscopy can assess corneal sub-basal nerve alterations in diabetic patients with DE. In-depth nerve grading can be used to track ocular pain and pathophysiological conditions of the ocular surface in diabetes patients with DE. Confocal corneal microscopy may have clinical utility as a rapid and objective test for assessing corneal neuropathic pain in diabetic patients with DE.

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Data availability statement Data are available upon reasonable request. The data used to support the findings of this study are available from the corresponding author upon request.

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