No Relation Between the Severity of Corneal Nerve, Epithelial, and Keratocyte Cell Morphology With Measures of Dry Eye Disease in Type 1 Diabetes

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Purpose. Patients with diabetes have a propensity to develop dry eye symptoms (DES), with reduced tear secretion and corneal sensitivity. The underlying pathologic basis of DES was explored in patients with Type 1 diabetes.

Methods. Forty-two patients with Type 1 diabetes mellitus (T1DM) (age: 49.21 ± 2.53 years, duration of diabetes: 29.98 ± 2.64 years) and 25 control subjects (age: 48.70 ± 2.84 years) underwent assessment of DES using a validated dry eye questionnaire, and tear stability and tear production were assessed using tear breakup time (TBUT) and Schirmer's test, respectively. Corneal confocal microscopy was undertaken to quantify corneal nerve fiber density (CNFD), branch density (CNBD), fiber length (CNFL), keratocyte density (KD), and corneal epithelial basal cell (CEBC) density and area.

Results. The prevalence of DES was significantly higher ($P = 0.03$), and TBUT ($P = 0.006$), corneal sensation ($P < 0.0001$), CNFD ($P = 0.001$), CNBD ($P = 0.001$), CNFL ($P = 0.003$), and KD ($P = 0.04$) were significantly lower in patients with T1DM compared to control subjects. However, these measures did not differ significantly between T1DM patients with and without dry eye. There was no correlation between DES and TBUT or corneal nerve keratocyte and CEBC morphology.

Conclusions. DES and TBUT are significantly increased in patients with T1DM, but are not related to corneal nerve, basal epithelial, or keratocyte cell morphology.

Keywords: dry eye, type 1 diabetes mellitus, corneal confocal microscopy, corneal epithelial basal cells, keratocytes, corneal nerves

The most common eye complication of diabetes is retinopathy; however, several additional complications including cataract, glaucoma, refractive alterations, and dry eye contribute to the morbidity of diabetic eye disease.[1,2] Indeed, dry eye has a reported prevalence of ~54.3% and is associated with an increased risk of epithelial defects and corneal ulcers in patients with diabetes.[3–5] Diabetic keratopathy can manifest as recurrent corneal erosions, ulceration, and delayed wound healing after surgery,[6] superficial punctate keratopathy, and neurotrophic keratitis.[5,7] Early manifestations of diabetic keratopathy include eye discomfort,[8] reduced corneal sensitivity,[9] and decreased tear stability[9,10] and secretion.[11,12] The International Dry Eye Workshop defined dry eye as a "multifactorial disease of the tears and ocular surface that result in symptoms of discomfort and visual disturbance and tear film instability with potential damage to the ocular surface and is accompanied by increased osmolality of the tear film and inflammation of the ocular surface."[13]

Multiple risk factors may predispose to dry eye symptoms (DES), with diabetes being a major risk factor.[14] While the exact underlying mechanisms for dry eye are unclear in diabetes, dry eye syndrome correlates with hemoglobin A1c (HbA1c) levels[4] and may also be associated with peripheral neuropathy and abnormal tear secretion.[15,16] DES was diagnosed in 76.5% of subjects with diabetic neuropathy compared to 44.4% in subjects without diabetic neuropathy.[8] Some studies suggest that denervation of the lacrimal gland and the accessory palpebral gland may reduce tear film reflex secretion in diabetes.[9,17] Lacrimal gland innervation is derived from the ophthalmic branch of the trigeminal nerve, and stimulation of trigeminal nerve receptors in the cornea mediates activation of these glands.[18] Hence corneal nerve degeneration may lead to a reduction in corneal nerve sensitivity,[19] reduced reflex-induced lacrimal gland secretion, and dry eye.[20] A reduction in corneal sensitivity may also cause increased evaporation of tears due to a reduced blink rate.[13] These abnormalities may lead to a reduction in corneal epithelial cell densities and further contribute to a loss of corneal integrity in diabetes.[21]

Within the cornea, stromal keratocytes play an important role in maintaining corneal stability and transparency by releasing cytokines and growth factors[22–24] and also contribute to corneal nerve regeneration.[25] Studies have demonstrated abnormal hyperreflectivity of keratocytes in patients with Sjogren's syndrome and dry eye[26,27] and increased density of

activated keratocytes in patients with dry eye due to Graves' orbitopathy.[28] However, few studies have assessed keratocyte density (KD) and morphology in diabetic patients with dry eye.[29,30]

In vivo corneal confocal microscopy (IVCCM) demonstrates significant abnormalities in corneal nerve, epithelial, and endothelial cell morphology in patients with Type 2 diabetes.[31] Patients with dry eye, with and without primary Sjogren's syndrome, show a reduction in superficial epithelial cell density but no change in corneal subbasal and stromal nerve density or basal epithelial cell density.[32] In a recent study of 53 patients with Type 1 diabetes mellitus (T1DM), an abnormality in tear film stability, secretion, and lipid layer quality was related to corneal sensitivity and the severity of somatic neuropathy, but corneal structure was not evaluated.[33] In a larger study of 243 patients with Type 2 diabetes, dry eye was related to retinopathy, but not neuropathy, and corneal structure was not assessed.[34]

We have undertaken a detailed evaluation of DES in relation to tear function, corneal nerve, basal epithelial cell, and stromal keratocyte cell morphology in patients with T1DM.

Materials and Methods

Study Subjects

This study was approved by the North Manchester Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki. Forty-two participants with T1DM (age: 49.21 ± 2.53 years) and 25 age-matched control subjects (age: 48.70 ± 2.84 years) were studied. Informed written consent was obtained from all participants. Exclusion criteria included any cause of neuropathy other than diabetes, including malignancy, connective tissue or infectious disease, deficiency of vitamin B12 or folate, chronic renal failure, liver failure, and active diabetic foot ulceration. In addition, participants with Graves' disease, rheumatoid arthritis, or a history of wearing contact lenses, laser treatment, ocular trauma, or those who worked in a dusty environment were excluded. Patients and controls did not report the use of any medication to treat dry eye.

Clinical and Ophthalmic Assessment

All participants underwent assessment of body mass index (BMI), HbA1c, lipid profile, and neuropathy using the neuropathy disability score (NDS). Dry eye symptoms were recorded and scored using question 7 of the Dry Eye Questionnaire (DEQ),[35] which includes five subquestions regarding the presence, severity, and frequency of DES (see Supplementary Fig. S1). In question 1, patients were asked about the five main symptoms of dry eyes, and in questions 2 to 5, they were asked about the intensity and frequency of these symptoms on a numeric rating scale ranging from 0 to 5, that is, from no to very intense symptoms. Dry eye was determined on the basis of a history of ocular discomfort, including a burning sensation, itchiness, gritty sensation, redness, and excessive tearing, based on the questionnaire. Patients with at least two out of six symptoms were considered to have dry eye.

All participants underwent general ocular surface and eyelid examination using slit-lamp biomicroscopy (Slit Lamp BC 900; Haag-Streit UK, Harlow, UK) to evaluate the presence of other ocular complications. Corneal sensitivity was assessed using a noncontact corneal aesthesiometer (NCCA) (Glasgow Caledonian University, Glasgow, Scotland, UK).[19]

The standard tear breakup time (TBUT) was assessed by applying moistened fluorescein strips to the conjunctival sac with minimal stimulation. The subject was then asked to gently blink five times to make sure that the fluorescein mixed adequately with the tear film. The time between the last blink and the appearance of the first corneal black spot in the tear film was used to define the TBUT and was averaged from three readings.[36] The Schirmer I test was performed without local anesthetic using a standard Schirmer strip (Dina strip Schirmer-Plus; GECIS, Villemorant, France) hooked for 5 minutes on the lateral one-third of the lower lid and quantified by measuring the length of wet strip in millimeters.[10]

Corneal Confocal Microscopy

All study subjects were scanned with a laser IVCCM (Heidelberg Retinal Tomograph III Rostock Cornea Module HRT III RCM (Heidelberg Engineering GmbH, Heidelberg, Germany) in the center of the cornea using a section mode according to our previously published protocol.[37]

Image Analysis

Six good-quality images from the center of the cornea (three from each eye) were selected following our protocol for image selection.[38] Image analysis was performed using our previously validated purpose-designed fully automated software (ACCMetrics; The University of Manchester, Manchester, UK).[39] Corneal nerve morphologic parameters included nerve fiber density (CNFD), the number of main nerve fibers/mm^2; nerve branch density (CNBD), the number of branch points on the main nerves/mm^2; and nerve fiber length (CNFL), the total length of nerves mm/mm^2. Two high-resolution images of the basal cell layer immediately anterior to the subbasal nerve layer were captured. The AlConfocal Rapid Image Evaluation System, 2-dimensional version (ARIEs 2D) (Alcon Research Ltd, Fort Worth, TX, USA),[40] was used in the manual mode to quantify corneal epithelial basal cell density (CEBCD) and area (CEBCA). Two high-resolution anterior, mid, and posterior stromal images (three per eye) were selected for KD analysis. The very first high-quality image after Bowman's layer was selected for the anterior stroma and the very first high-quality image before Descemet's layer was selected for the posterior stroma with an image between the anterior and posterior stroma being selected for the midstroma.

Keratocyte cells were manually quantified using purpose-designed manual software (CCMetrics; The University of Manchester) and were identified as hyperreflective cells against the dark background in the stroma.[41] Mean KD was counted as the number of cells per square millimeter (cells/mm^2) in the anterior, mid, and posterior stroma.

Statistical Analysis

Statistical analysis was undertaken using IBM SPSS v19.0 (Chicago, IL, USA). All the data were expressed as mean ± SE and analysis included descriptive and frequency statistics. To test for statistical differences, independent sample *t*-tests (Mann-Whitney *U* test for nonparametric) were used between the two groups and 1-way ANOVA with Bonferroni adjustment was used among groups. Correlations were measured by calculating the Pearson correlation coefficient (Spearman for nonparametric) for continuous variables and Pearson's χ^2 test of independence and a Fisher's exact test for categorical variables. For all comparisons $P < 0.05$ was considered to be significant.

Table 1. Clinical, Demographic, and Ophthalmic Characteristics in Patients With T1DM and Age-Matched Control Subjects

Parameters	Controls	T1DM
Number	25	42
Age, y	48.70 ± 2.84	49.21 ± 2.53
Sex (female/male)	(14/11)	(15/27)
Ethnicity (Asian/European)	(4/21)	(5/37)
Duration of diabetes, y	0	29.98 ± 2.64*
Smoking, cigarettes/d	0.4 ± 2	1.63 ± 4.3
HbA1c, mmol/mol	34.56 ± 0.82	69.03 ± 3.31*
BMI	26.78 ± 1.16	28.01 ± 0.74
NDS, 0–10	0.48 ± 0.25	2.28 ± 0.47†
Retinopathy	0	38.1%, 16/42*
Laser treatment	0	21.4%, 9/42*
Prevalence of dry eye, %	24	50§
Eye dryness symptoms, 0–6	0.61 ± 0.26	1.26 ± 0.25
Frequency of eye dryness symptoms, 0–4	1 ± 0.26	0.87 ± 0.18§
Intensity of dryness in the morning, 0–5	0.26 ± 0.12	1.03 ± 0.23§
Intensity of dryness in the afternoon, 0–5	0.22 ± 0.15	0.61 ± 0.20
Intensity of disturbance, 0–5	0.30 ± 0.15	1.26 ± 0.26§
TBUT, s	8.5 ± 1.08	5.39 ± 0.4‡
Schirmer test, mm	10.7 ± 1.64	9.27 ± 1.36

All data are presented as mean ± SE. All symbols represent statistically significant differences compared with controls.

* $P < 0.0001$.

† $P = 0.004$.

‡ $P = 0.006$.

§ $P < 0.05$.

Results

Clinical and Peripheral Neuropathy Assessment

Forty-two patients with T1DM (age 49.21 ± 2.53; duration of diabetes 29.98 ± 2.64 years) and 25 healthy control subjects (age 48.70 ± 2.84) were assessed. There were no significant differences in age, sex, ethnicity, BMI, or smoking history between patients with T1DM and controls (Table 1). HbA1c ($P < 0.0001$), NDS ($P = 0.004$), prevalence of retinopathy ($P < 0.0001$), and previous laser treatment ($P < 0.0001$) were significantly greater in patients with T1DM (Table 1).

Dry Eye Assessment

The prevalence (50% vs. 24%, $P = 0.03$), frequency ($P = 0.02$), and intensity ($P = 0.02$) of DES, particularly in the morning, were significantly higher in patients with T1DM compared to controls (Table 1). Patients with T1DM demonstrated a significantly lower TBUT ($P = 0.006$), but no difference in the Schirmer test compared to controls.

Table 2. Corneal Nerve, Corneal Epithelial Cell, and Keratocyte Morphology in Patients With T1DM and Age-Matched Healthy Controls

Parameters	Controls	T1DM
NCCA, mb	0.46 ± 0.08	1.15 ± 0.16*
CNFD, no./mm^2	27.34 ± 0.96	21.21 ± 1.56†
CNBD, no./mm^2	38.25 ± 2.82	25.64 ± 2.20†
CNFL, mm/mm^2	16.04 ± 0.57	13.23 ± 0.69‡
KD, no./mm^2	434.24 ± 6.17	397.52 ± 11.57§
CEBC density, no./mm^2	7492.29 ± 123	7402.25 ± 75.07
CEBC area, μm^2	134.50 ± 2.19	136.17 ± 1.40

All data are presented as mean ± SE. All symbols represent statistically significant differences compared with controls.

* $P < 0.0001$.

† $P = 0.001$.

‡ $P = 0.003$.

§ $P = 0.04$.

In Vivo Corneal Confocal Microscopy and Corneal Sensation

Corneal sensation threshold was significantly ($P < 0.0001$) higher and CNFD ($P = 0.001$), CNBD ($P = 0.001$), CNFL ($P = 0.003$) and KD ($P = 0.04$) were significantly lower in patients with T1DM compared to control subjects (Table 2). CEBC density and area did not differ significantly between patients with T1DM and controls and did not correlate with age, sex, HbA1c, duration of diabetes, or corneal nerve morphology.

T1DM Patients With and Without Dry Eye

T1DM patients without dry eye had a significant reduction in TBUT ($P = 0.02$) and CNBD ($P = 0.05$) compared to healthy control subjects. However, when comparing T1DM patients with and without dry eye there was no significant difference in corneal sensitivity, corneal nerve morphology, or CEBC density or area (Fig.; Table 3). A significant inverse correlation ($r = -0.53$, $P = 0.02$) was observed between TBUT and HbA1c, but not with corneal nerve morphology or CEBC density and size.

KD was significantly reduced in patients with T1DM compared to healthy controls ($P = 0.04$), but did not differ between patients with and without dry eye. Mean KD showed a significant inverse correlation with NDS ($r = -0.51$, $P = 0.003$) and HbA1c ($r = -0.4$, $P = 0.05$).

Discussion

In the present study we have shown an increased prevalence and severity of dry eye disease and a significantly lower TBUT in patients with T1DM, consistent with a previous study.[33] Our study also shows an inverse correlation between TBUT and poor glycemic control, in agreement with the studies by Ozdemir et al.[17] and Najafi et al.[34] However, our study shows no significant difference in TBUT and Schirmer's test in diabetic patients with and without symptoms of dry eye, confirming a poor correlation between tests of dry eye and symptoms.[42]

Experimental and clinical studies consistently report structural, functional, and metabolic alterations in the corneas of patients with diabetes.[4,16,21,37,43–45] Indeed it has been postulated that diabetic keratopathy is related to corneal denervation and epithelial cell alterations.[7] However, few human studies have directly related DES with structural and functional alterations in the cornea. We have previously used IVCCM to show significant corneal nerve, epithelial, endothelial, and keratocyte cell abnormalities in patients with diabetes.[31,46–48] In the present study we also show a significant reduction in CNFD, CNBD, and CNFL. Corneal nerves play an important role in maintaining the anatomic integrity of the ocular surface and supply trophic factors that maintain epithelial cell morphology.[5] In a recent experimental study, a reduction in the length of the subbasal nerve plexus was associated with a reduction in corneal epithelial cell density.[49] However, these findings have not been translated to patients, as Chang et al.[50] and Ishibashi et al.[44] have reported no association between CEBC density and corneal nerve morphology in Type 2 diabetes mellitus (T2DM) patients. In the present study we also demonstrate no association between CEBC density or area and corneal nerve morphology and no difference between T1DM patients with and without dry eye.

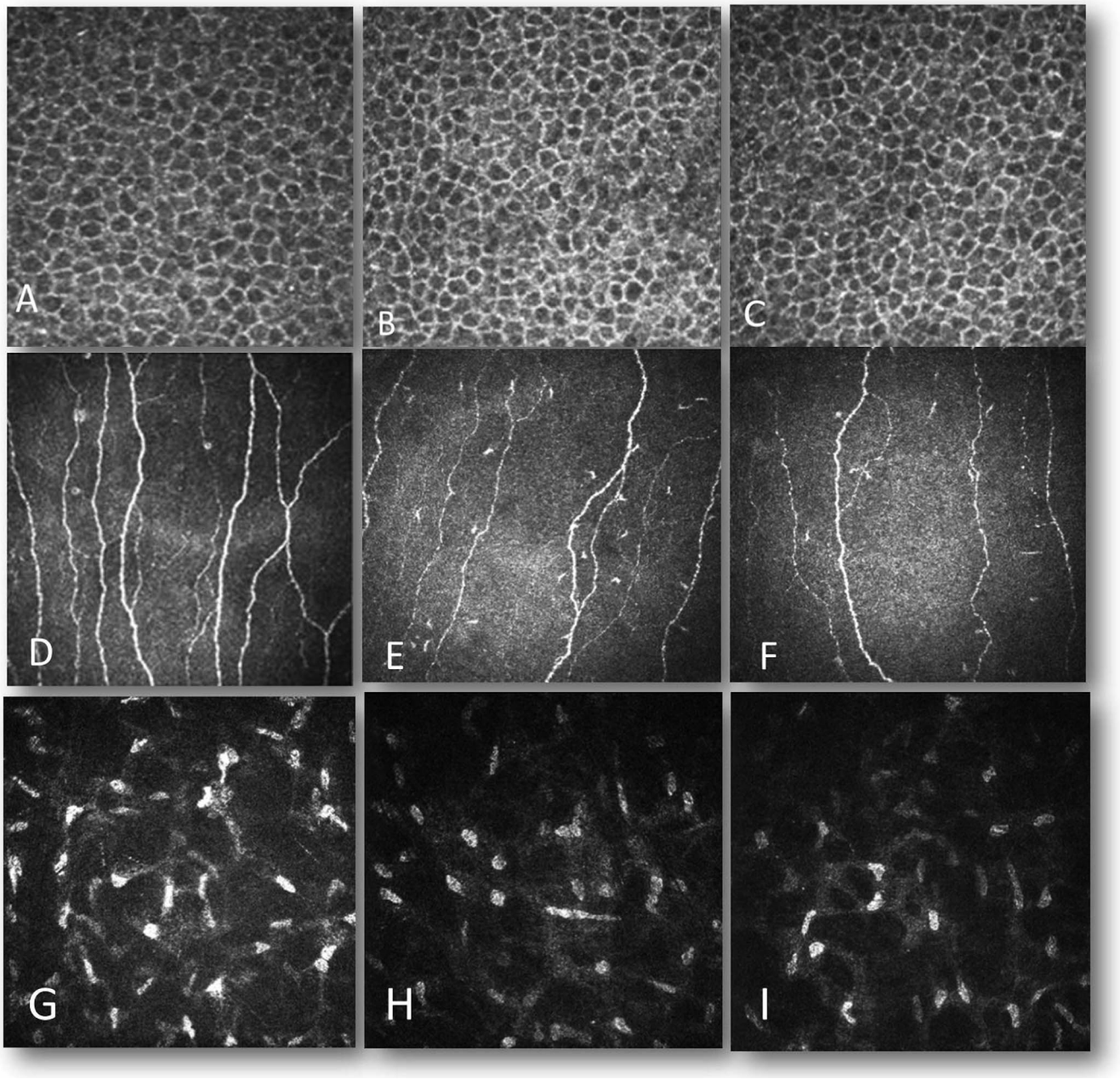

FIGURE. Corneal confocal microscopy images of corneal epithelial basal cells, subbasal nerves, and keratocytes in a control subject (**A**, **D**, **G**), a patient with T1DM without dry eye (**B**, **E**, **H**), and a patient with T1DM and dry eye (**C**, **F**, **I**), respectively.

Benitez del Castillo et al.[32] also observed no significant change in the density of CEBCs in patients with and without dry eye syndrome. A reduction in corneal nerve morphology has been proposed to lead to reduced corneal sensitivity and lacrimal gland dysfunction with reduced tear production and tear stability. In the present study we show no association between corneal sensitivity and corneal nerve abnormalities with subjective symptoms of dry eye or tear stability and production.

In the present study we also show a significant reduction in keratocyte cell density in patients with T1DM, in agreement with previous studies.[31,51] We also show a significant correlation between KD and HbA1c, which could be due to an accumulation of advanced glycation end products and keratocyte apoptosis.[52,53] However, there was no significant difference in T1DM patients with and without dry eye.

A limitation of the current study is the subjective nature of a self-reported questionnaire to evaluate dry eye. Indeed, previous studies have shown a poor relationship between objective tests like the Schirmer test and TBUT with self-reports.[54,55] The evaluation of tear composition may provide insights into the underlying mechanisms of dry eye disease, but to date has been limited to T2DM. A small tear proteomic study in eight patients with T2DM and dry eye syndrome showed increased expression of apoptosis-related proteins, immunity-, and inflammation-related proteins as well as glycometabolic proteins.[56] A recent study has shown increased levels of tear insulin-like growth factor binding protein-3, which correlated with corneal nerve fiber length and branch density in patients with T2DM, but there was no difference between patients with and without dry eye.[57]

Table 3. Clinical and Demographic Characteristics, Corneal Nerve, Corneal Basal Epithelial, and Keratocyte Cell Morphology in T1DM Patients With and Without Dry Eye Compared to the Healthy Controls Without Dry Eye

Parameters	Controls No Dry Eye	T1DM No Dry Eye	T1DM Dry Eye
Number	19	23	19
Age, y	45.8 ± 2.91	49.97 ± 3.56	48.3 ± 3.65
Smoking, cigarettes/d	0.53 ± 2.2	0.96 ± 3.3	2.50 ± 5.3
HbA1c, mmol/mol	33.54 ± 0.7	67.90 ± 4.37*	70.53 ± 5.23*
Duration of diabetes, y	0	29.49 ± 3.90	30.56 ± 3.53
Retinopathy, %	0	30.4	47.4
Laser treatment, %	0	21.7	47.4
TBUT, s	8.81 ± 1.09	5.3 ± 0.37‡	5.4 ± 0.65†
Schirmer test, mm	10.18 ± 1.50	10.43 ± 2.37	8.35 ± 1.62
NCCA, mb	0.43 ± 0.07	0.99 ± 0.15	1.34 ± 0.31†
CNFD, no./mm^2	27.37 ± 1.2	22.15 ± 2.33	20.62 ± 2.02§
CNBD, no./mm^2	38.88 ± 3.5	26.88 ± 3.22§	24.12 ± 2.95†
CNFL, mm/mm^2	16.07 ± 0.73	13.49 ± 1.05	12.92 ± 0.87
KD, no./mm^2	433.12 ± 6.64	417.11 ± 11.31‡	382.63 ± 20.07†
CEBC density, no./mm^2	7409.16 ± 129.6	7327.5 ± 97.4	7493.6 ± 116.2
CEBC area, μm^2	135.94 ± 2.49	137.5 ± 1.85	134.56 ± 2.151

All data are presented as mean ± SE. All symbols represent statistically significant differences compared with controls.
* $P < 0.0001$.
† $P = 0.01$.
‡ $P = 0.02$.
§ $P = 0.04$.

In conclusion, while corneal nerve, KD, and CEBC morphological abnormalities occur in patients with T1DM, they do not relate to the occurrence or severity of dry eye. This suggests that these abnormalities may occur independent of each other or that younger patients with T1DM may differ from older T2DM patients. Nevertheless, given the severe consequences of diabetic keratopathy, further studies exploring the underlying basis of this abnormality are warranted.

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