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Early central and peripheral corneal microstructural changes in type 2 diabetes mellitus patients identified using in vivo confocal microscopy

A case-control study

Jing-hao Qu, PhD, Lei Tian, PhD, Xiao-yu Zhang, MD, Xu-guang Sun, PhD*

Abstract

The aim of this study was to find early central and peripheral corneal microstructural changes in healthy subjects and type 2 diabetes mellitus (T2DM) patients with/without cornea fluorescein dot staining.

This is a prospective case-control study of T2DM patients with/without cornea fluorescein dot staining. Age, sex, duration of diabetes, and serum glycosylated hemoglobin A1c (HbA1c) levels were recorded. Keratograph 5M (K5M) and in vivo confocal microscopy were performed on all subjects. The cornea was divided into 5 zones: central, superior, temporal, nasal, and inferior. Basal epithelial cell (BEC) density, the area of BEC, sub-basal nerve plexus (SBN) density, Langerhans cell (LC), and endothelial cell (EC) density were quantitatively analyzed.

This study included a total of 87 individuals (28 males and 59 females; mean age, 62.30 ± 9.93 years) with T2DM, without ($n=48$; T2DM group 1) and with ($n=39$; T2DM group 2) cornea fluorescein staining, as well as 51 age- and sex-matched healthy subjects (18 males and 33 females; mean age, 61.53 ± 10.15 years). Ocular Surface Disease Index scores, Schirmer I test, tear meniscus height, the first breakup of tear film occurrence (NIKBUT-first), and the average time of all breakup incidents (NIKBUT-average) values were significantly lower for the T2DM groups than for the healthy group. The corneal sensations of all cornea positions in the T2DM groups were significantly different from the control group. The HbA1c in the T2DM groups showed a negative correlation with central BEC density ($R=0.348$, $P=.015$; $R=0.91$, $P=.001$); there was no correlation of HbA1c with BEC density in the control group. The BEC density, the area of BEC, SBN, and LC density of T2DM group 1 and T2DM group 2 were significantly different compared with the control group in all corneal positions ($P<.001$). The BEC density of T2DM group 2 was significantly different from T2DM group 1 in the central ($P=.044$) and inferior ($P=.013$) zones. The area of BEC in T2DM group 2 was significantly different from T2DM group 1 in inferior zone ($P=.014$) and other corneal positions showed was no significant difference ($P>.05$). The SBN density of T2DM group 2 was not significantly different from T2DM group 1 in all corneal positions ($P>.05$). The LC density of T2DM group 2 was significantly different from T2DM group 1 in the central ($P=.006$) and inferior ($P=.006$) zones. Although the LC density in the T2DM groups showed no significant difference in all corneal zones ($P>.05$), the LC density in the central zone was significantly lower compared with the peripheral zone in the control group ($P=.001$). The central ECs in the 3 groups were not significantly different ($P>.05$).

LC induced an immune-mediated contribution to corneal nerve damage and may influence the early stages of BEC proliferation and differentiation in T2DM. BEC density was the reliable index for evaluating the early condition of diabetic corneal epitheliopathy. The BEC density of the central and inferior corneal zones was more sensitive.

Abbreviations: AGEs = advanced glycation end products; BEC = basal epithelial cell; DCs = dendritic cells; DR = diabetic retinopathy; IVCM = in vivo confocal microscopy; K5 M = keratograph 5 M; LC = Langerhans cell; NIKBUT-average = average time of all breakup incidents; NIKBUT-first = first breakup of tear film occurs; SBN = sub-basal nerve plexus; T2DM = Type 2 diabetes mellitus; TMH: tear meniscus height.

Keywords: basal epithelial cell, diabetic corneal epitheliopathy, endothelial cell, Langerhans cell, subbasal nerve plexus, type 2 diabetes mellitus

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1. Introduction

Diabetes is one of the most common metabolic disorders in the world and the prevalence of diabetes in adults has been increasing in recent decades.[1,2] An estimated 382 million people were reported to have type 2 diabetes mellitus (T2DM) in 2013 and this figure is projected to increase by 55% to 592 million by 2035.[3] Although diabetic retinopathy (DR) is the most common ocular complication and a leading cause of blindness worldwide, the ocular surface can also be affected.[4] Corneal findings of T2DM patients include punctate keratitis, recurrent corneal erosions, delayed corneal epithelial healing, persistent epithelial defects, corneal ulcers, and reduced corneal sensitivity.[5–7]

The effects of T2DM on epithelial basal cell, the epithelial basement membrane, and sub-basal nerve plexus (SBN) can lead to epitheliopathy and corneal adhesion disorders. Basement membrane changes in T2DM can affect the corneal epithelium and predispose patients to infections. Such changes include a reduction of cornea basal epithelial cell (BEC) as well as an increase in thickness and irregularity of the corneal epithelial basement membrane.[8,9] The cornea BECs are located immediately above Bowman membrane. BECs present as brightly bordered cells in which the cell nucleus is not visible.[10] Much research has reported that BEC density was significantly lower in the cornea of people with T2DM compared with controls.[8,11] Decreases in BEC density may be associated with hemoglobin A1c (HbA1c) and advanced glycation end products (AGEs).[12] Corneal nerve density measured as total nerve length has emerged as a more reliable measure of nerve density compared with nerve branch density or tortuosity.[6] T2DM affects the corneal SBN, leading to a reduction in SBN density.[13] The dominant antigen-presenting cells in the cornea and ocular surface are Langerhans cell (LC) and dendritic cells (DCs).[14] It is of interest that using in vivo confocal microscopy (IVCM) had shown an increase in the number of highly reflective cells, or "presumably DCs," in the cornea of patients with diabetes.[15]

IVCM has become the standard tool for assessing the living cornea at the cellular level in diseased and healthy subjects.[6] The instrument shows excellent repeatability and reproducibility in the evaluation of the corneal epithelium, SBN, and LC density, in both healthy controls and in people with T2DM.[15–17]

BEC and SBN density were lower in T2DM patients, Furthermore, LC density was increased. Our aim was to determine changes in the corneal epithelium, as observed by IVCM, can be used to assess the epithelium statement in the early stages of diabetic keratopathy.

2. Materials and methods

2.1. Study subjects

We studied 87 individuals (28 males and 59 females; mean age, 62.30 ± 9.93 years) with T2DM, without (n=48; T2DM group 1) and with (n=39; T2DM group 2) cornea fluorescein staining, as well as 51 age- and sex- matched healthy subjects (18 males and 33 females; mean age, 61.53 ± 10.15 years). In both patients with T2DM and control subjects, 1 eye was randomly selected for measurement and statistical analysis. For this study, T2DM subjects were diagnosed based on the standards of medical care in diabetes.[18] Potential subjects were excluded from the study if they had undergone previous corneal or had ocular surgery, had any ocular pathology other than DR and keratopathy, or chronic use of topical ocular medications. Patients with T2DM were divided into those with corneal fluorescein staining scores of 0 (48 patients; mean age, 60.51 ± 8.37 years) and −5 points (39 patients; mean age, 63.75 ± 10.91 years). Data were collected from March 2015 to November 2016 in Beijing Tongren Hospital, Beijing, China. All participants signed an informed consent form in accordance with the tenets of the Declaration of Helsinki and this study was approved by the institutional review board of Beijing Tongren Hospital, Beijing, China.

2.2. Measurements

2.2.1. Ocular examinations. Each patient was asked to complete the Ocular Surface Disease Index (OSDI) questionnaire (range: 0–100). In all eyes, an ophthalmic examination was performed in the same order as follows: first, medical history, visual acuity, fundus photography, and intraoculaer pressure measurement; second, tear meniscus height (TMH) measurement and then NIKBUTs measurement with Keratograph 5 M; third, corneal and conjunctiva fluorescein staining, nonanesthetized Schirmer I test, and corneal sensation measured with the Cochet-Bonnet esthesiometer; finally, BEC, SBN, LC, stroma, and endothelium density measured with IVCM.

2.2.2. Keratograph 5 M measurement. All subjects underwent imaging with the Keratograph 5 M (K5 M; Oculus Optikgerate GmbH, Wetzlar, Germany) equipped with a modified tear film scanning function. In each subject, inferior TMH images were captured and measured perpendicular to the lid margin at the central point relative to the pupil center using an integrated ruler. NIKBUT was measured as the time in seconds between the last complete blink and the first perturbation of placid rings projected onto the surface of the cornea, which the device automatically detects.[19] K5 M generated 2 measures for NIKBUT: the time at the first breakup of tear film occurs (NIKBUT-first) and the average time of all breakup incidents (NIKBUT-average).

2.2.3. Corneal and conjunctival staining scores. Corneal and conjunctiva staining was evaluated under a yellow filter after instillation of fluorescein. The grading system recommended by the NEI Workshop on Clinical Trials in Dry Eyes[20] divides the cornea into 5 zones: central, superior, temporal, nasal, and inferior as shown in Figure 1. For each zone, the amount of corneal fluorescein staining was graded on a scale of 0 to 3: 0 = normal or negative slit-lamp findings; 1 = mild or superficial stippling; 2 = moderate or punctate staining, including superficial abrasion of the cornea; and 3 = severe abrasion or corneal

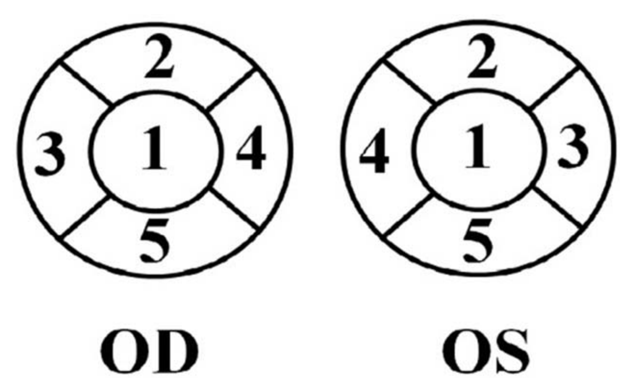

Figure 1. Diagram of the division of the corneal surface for measuring fluorescein, sensation and in vivo confocal microscopy. The 5 corneal zones include the following: 1 = central; 2 = superior; 3 = temporal; 4 = nasal; 5 = inferior.

erosion, deep corneal abrasion, or recurrent erosion. The maximum score is 15.

2.2.4. Schirmer I test. The Schirmer I test is a useful assessment of aqueous tear production. In this experiment, the inferior conjunctival fornix was dried with a cotton stick. One minute later, a standard 5×40 mm Schirmer test strip was placed over the junction of the middle and outer third of the inferior lid. The patients were instructed to keep their eyes closed during the test. The test lasted 5 minutes, and the amount of wetting was recorded.

2.2.5. Cornea esthesiometer. Cochet-Bonnet esthesiometer (Luneau, Prunay-Le-Gillon, France) was used to measure corneal sensation. The nylon monofilament had a diameter of 0.12 mm and a fully extended length of 60 mm. The central, superior, inferior, nasal, and temporal cornea were touched once on each eye, beginning at a filament length of 60 mm. If a positive response was not detected, the filament length was shortened in 5-mm steps each time and the procedure repeated until there was a positive response. Corneal sensation was calculated as the mean obtained from the 5 corneal areas on each eye (Fig. 1).

2.2.6. IVCM and ImageJ. All patients underwent IVCM using IVCM (Heidelberg Retina Tomograph III Rostock Cornea Module HRT III RCM; Heidelberg Engineering GmbH, Heidelberg, Germany). Genteal Gel (0.2% carbomer eye drops; Dr. Gerhard Mann, Chem.-Pharm, Fabrik GmbH) was applied in a disposable sterile polymethylmethacrylate cap (Tomo-Cap; Heidelberg Engineering GmbH), which was placed on the tip of the objective lens. A drop of local anesthetic (0.4% tetracaine hydrochloride) was administered and the subject was asked to fixate on a distant target before commencing scanning of the central cornea. The procedure took approximately 10 minutes to perform and none of the subjects complained of pain, inconvenience, or visual disturbance. All examinations were performed by a single experienced and blinded examiner. Section scans of the 5 corneal zones were performed (Fig. 1) and each zone was recorded with 6 positions using the Heidelberg HRT-III microscope, with 384×384 pixels and a field of view of $400 \times 400\,\mu m^2$. Six-good quality images (image quality >80) of the BEC, SBN, LC, stroma, and endothelium cells were selected for each zone and used for image analysis by the examiner. The average of 6 measurements was used for further comparative analysis.[21] BEC was defined as the first 3 clear scans anterior to Bowman layer and the posterior stroma was defined as the first 3 clear images immediately anterior to Descemet membrane. On average, Bowman layer was used to quantify nerve fiber morphology in all patients and controls and the average results of all these images were calculated. SBN density was defined as the total nerves lengths in units of millimeter per square millimeter.[22] BEC and endothelium cells density were measured manually using HRT III proprietary software. The area of BEC and LC density were analyzed using ImageJ (National Institutes of Health, Bethesda, MD). The SBN was analyzed using NeuronJ software (Erik Mitering). NeuronJ is an ImageJ plugin to facilitate the tracing and quantification of elongated image structures.[23]

2.3. Statistical analysis

Descriptive statistical results were described as the mean, standard deviation (SD), and 95% confidence interval. Statistical analysis was performed using SPSS 18.0 (SPSS for Windows, Chicago, IL) for Windows. The Kolmogorov-Smirnov test was used to check for a normal distribution of the quantitative data, which are here provided as the mean and SD. An analysis of variance or the Kreskas-Wallis test was applied for comparison among 3 groups. For continuous variables, Student t test was used for comparing 2 groups; for univariate analysis, the χ^2 test was used. For vicariate correlation analysis, Pearson correlation test or Spearman test was used. A P value <0.05 was considered statistically significant.

3. Results

3.1. Demographics

A total of 87 T2DM patients and 51 healthy subjects were recruited for the study. Patients' characteristics and clinical data are detailed in Table 1. There were no significant differences in the mean age ($F=1.232$, $P=.295$) or sex ($P=.749$) between patients with T2DM and controls. There was no conjunctival staining in all 3 groups. The NEI scores were significantly higher in T2DM group 2. OSDI scores, Schirmer I test scores, and TMH, NIKBUT-first, and NIKBUT-average values were significantly lower for the T2DM groups than for the healthy group (Table 1), whereas the same values were not significantly different between T2DM group 1 and T2DM group 2 ($P=.912$, $P=.835$, $P=.824$, $P=.647$, $P=.626$).

Table 1

Characteristics of the study population.

Parameters	T2DM group 1	T2DM group 2	Control group	P
Age, y	60.51 ± 8.37	63.75 ± 10.91	61.53 ± 10.15	.295
sex (male/female)	14/34	14/25	18/33	.749
HbA_{1c} (%)	7.70 ± 1.12	7.84 ± 1.75	5.48 ± 0.31	<.0001*
Duration of T2DM, y	13.40 ± 8.30	13.90 ± 5.20	0	<.0001*
OSDI (scores)	30.45 ± 15.31	31.45 ± 16.31	3.84 ± 6.90	<.0001*
NEI scores	0	2.97 ± 1.58	0.03 ± 0.18	<.0001*
Schirmer test (mm/5 min)	6.83 ± 4.29	6.67 ± 4.37	13.78 ± 2.26	<.0001*
TMH, mm	0.22 ± 0.04	0.23 ± 0.06	0.27 ± 0.11	.001*
NIKBUT-first, s	5.62 ± 2.79	5.27 ± 2.41	7.38 ± 4.96	.013*
NIKBUT-average, s	7.76 ± 3.81	7.29 ± 3.50	10.34 ± 5.55	.002*

NEI scores = National Eye Institution workshop scores, NIKBUT = noninvasive keratograph tear breakup time, OSDI = Ocular Surface Disease Index, T2DM = type 2 diabets mellitus, TMH = tear meniscus height. Mean ± SD (95% CI). χ^2 test was used in sex; Kruskal-Wallis test was used in OSDI and NEI scores; other values were calculated using 1-way analysis of variance.
* $P<.01$.

Table 2

Corneal Cellular changes in different corneal positions.

Corneal zones		Control group (A)	T2DM group 1 (B)	T2DM group 2 (C)	*P*			
					Total	A vs. B	A vs. C	B vs. C
1	Corneal sensation, cm	5.98±0.10	5.47±0.42	4.91±0.81	<.001	<.001*	<.001†	<.001‡
	BEC density, cell/mm^2	5980±420	5013±318	4848±381	<.001	<.001*	<.001†	.044§
	Area of BEC, μm^2	117.2±17.6	137.6±25.9	145.2±30.1	<.001	<.001*	<.001†	.254
	SBN density, mm/mm^2	20.95±2.69	10.99±2.38	10.99±1.71	<.001	<.001*	<.001†	.831
	LC density, cell/mm^2	24.29±5.13	52.06±16.29	63.67±20.66	<.001	<.001*	<.001†	.006‡
	EC density, cell/mm^2	2597±306	2527±325	2529±333	.446	.237	.909	.330
2	Corneal sensation, cm	5.97±0.12	5.49±0.43	4.74±0.80	<.001	<.001*	<.001†	<.001‡
	BEC density, cell/mm^2	6115±391	4868±263	4872±267	<.001	<.001*	<.001†	.976
	Area of BEC, μm^2	114.9±21.6	143.9±25.4	144.1±23.5	<.001	<.001*	<.001†	.960
	SBN density, mm/mm^2	20.87±3.05	10.82±3.12	10.93±3.10	<.001	<.001*	<.001†	.828
	LC density, cell/mm^2	38.04±8.40	60.42±28.52	63.46±30.40	<.001	<.001*	<.001†	.590
3	Corneal sensation, cm	5.98±0.10	5.48±0.41	4.55±0.73	<.001	<.001*	<.001†	<.001‡
	BEC density, cell/mm^2	6115±303	4844±264	4821±255	<.001	<.001*	<.001†	.663
	Area of BEC, μm^2	120.3±20.3	145.3±30.5	141.6±29.5	<.001	<.001*	<.001†	.536
	SBN density, mm/mm^2	20.83±2.98	10.67±3.00	10.35±2.99	<.001	<.001*	<.001†	.588
	LC density, cell/mm^2	38.53±8.15	62.99±27.70	66.15±29.44	<.001	<.001*	<.001†	.616
4	Corneal sensation, cm	5.97±0.12	5.46±0.44	4.71±0.84	<.001	<.001*	<.001†	<.001‡
	BEC density, cell/mm^2	6115±300	4889±327	4888±330	<.001	<.001*	<.001†	.997
	Area of BEC, μm^2	119.5±19.1	145.4±26.0	147.0±24.7	<.001	<.001*	<.001†	.752
	SBN density, mm/mm^2	20.41±3.21	10.35±3.3	10.31±3.36	<.001	<.001*	<.001†	.983
	LC density, cell/mm^2	36.32±9.13	64.33±28.27	68.27±29.11	<.001	<.001*	<.001†	.474
5	Corneal sensation, cm	5.96±0.14	5.46±0.38	4.77±0.82	<.001	<0.001*	<.001†	<.001‡
	BEC density, cell/mm^2	6013±348	4799±307	4628±329	<.001	<.001*	<.001†	.013§
	Area of BEC, μm^2	119.1±19.4	152.1±30.6	164.8±31.9	<.001	<.001*	<.001†	.014§
	SBN density, mm/mm^2	20.35±3.22	10.24±3.27	9.90±3.28	<.001	<.001*	<.001†	.606
	LC density, cell/mm^2	37.94±9.54	65.10±35.14	76.33±38.00	<.001	<.001*	<.001†	.006‡

The BEC density in the central zone and the area of BEC in nasal zone were calculated using analysis of variance, others were calculated using Kruskal-Wallis test ($\alpha'=0.017$). BEC=basal epithelial cell, LC=Langerhans cell, SBN=sub-basal nerve plexus.

* $P<.01$.

† $P<.01$.

‡ $P<.01$.

§ $P<.05$.

3.2. Corneal sensation

The corneal sensations of all corneal positions in the T2DM groups were significantly different from the control group (Table 2). T2DM group 2 was significantly different from T2DM group 1 ($P<.01$).

3.3. BEC

The BEC density of T2DM group 2 was significantly different from T2DM group 1 in the central ($P_1=.044$) and inferior zones ($\alpha'=0.017$, $P_5=.013$). The BEC density of T2DM group 1 and T2DM group 2 was significantly different compared with the control group in all corneal positions ($\alpha'=0.017$, $P_{12345}<.001$, Fig. 2A$_{1-3}$ and Fig. 3).

3.4. The area of BEC

The area of BEC in T2DM group 2 was significantly different from T2DM group 1 in the inferior zone ($\alpha'=0.017$, $P_5=.014$), whereas other corneal positions were not significantly different ($P_1=.254$; $P_2=.96$; $P_3=.588$; $P_4=.752$). The area of BEC in T2DM group 1 and T2DM group 2 was significantly different compared with the control group among all corneal positions ($\alpha'=0.017$, $P_{12345}<.001$, Fig. 4).

3.5. SBN

The SBN density of T2DM group 2 was not significantly different from T2DM group 1 in all corneal positions ($\alpha'=0.017$, $P_1=.831$; $P_2=.828$; $P_3=.588$; $P_4=.983$; $P_5=.606$). The SBN in T2DM group 1 and T2DM group 2 was significantly different compared with the control group among all corneal positions ($\alpha'=0.017$, $P_{12345}<.001$, Fig. 2B$_{1-3}$ and Fig. 5).

3.6. LC

The LC density of T2DM group 2 was significantly different from T2DM group 1 in the central ($\alpha'=0.017$, $P_1=0.006$) and inferior zones ($\alpha'=0.017$, $P_5=0.006$). The LC density of T2DM group 1 and T2DM group 2 was significantly different compared with the control group in all corneal positions ($\alpha'=0.017$, $P_{12345}<.01$). Although the LC density in the T2DM groups showed no significant differences in all corneal zones ($F=1.735$, $P=.143$; $\chi^2=5.450$, $P=.244$), the LC density in the central zone was significantly lower compared with peripheral in the control group ($\chi^2=96.869$, $P=.001$, Fig. 2C$_{1-3}$ and Fig. 6).

3.7. Endothelial cell

The central endothelial cells in the 3 groups were not significantly different ($\chi^2=1.616$, $P=.446$, Table 2).

The HbA1c in the T2DM groups showed a negative correlation with central BEC density, but there was no correlation of HbA1c with BEC density in the control group. The density of BEC in all groups showed a positive correlation with the area of BEC. The density of BEC in the T2DM group showed a positive correlation with SBN density in all corneal zones except the temporal zone. The density of SBN in the T2DM

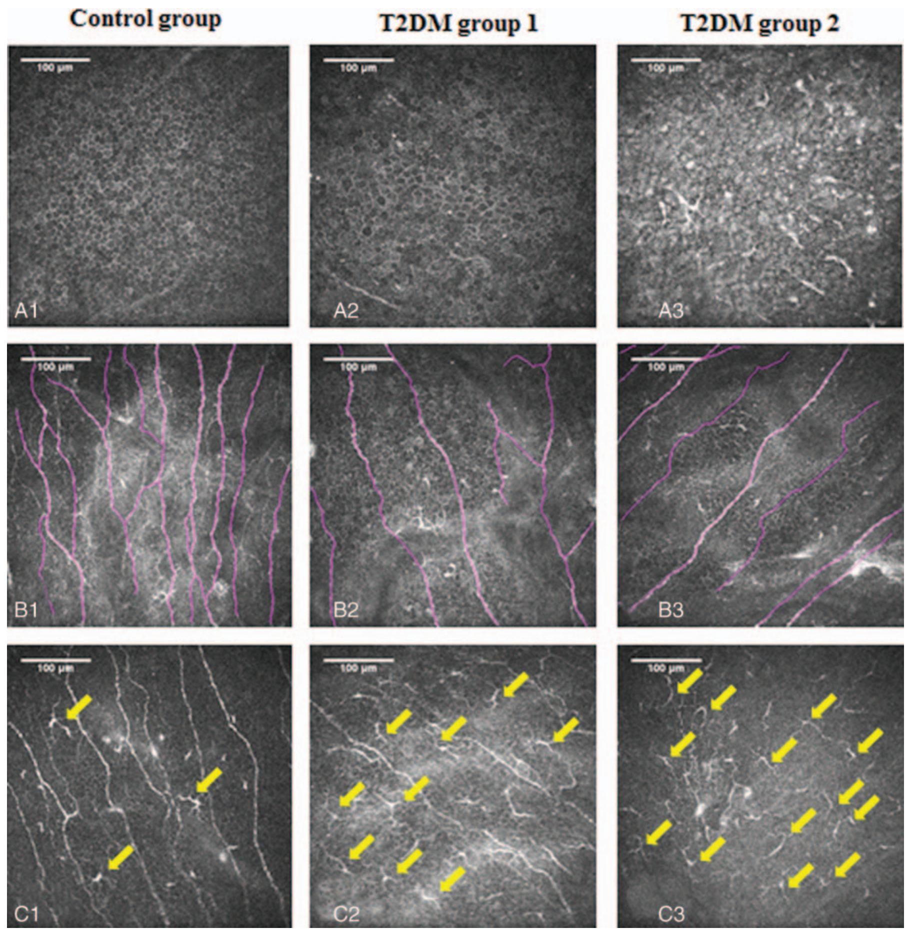

Figure 2. Basal epithelial cell was reduced in the T2DM groups (A2: group 1 and A3: group 2) compared with the control group (A1). Sub-basal nerve plexus was reduced in the T2DM groups (B2: group1 and B3: group2) compared with the control group (B1); the red lines show the sub-basal nerve plexus. LC was reduced in the T2DM groups (C2: group 1, and C3: group 2) compared with the control group (C1); the yellow arrows show the Langerhans cell. T2DM=type 2 diabetes mellitus.

group showed a negative correlation with LC density in all corneal zones except the superior zone. The BEC density in the T2DM groups showed a negative correlation with inferior LC density, but there was no correlation of BEC density with LC density in other positions in the T2DM groups (supplemental material, http://links.lww.com/MD/B854).

4. Discussion

T2DM patients typically complain of dry eye symptoms including burning and foreign body sensations. The Schirmer I test has shown lower tear production rates in people with diabetes than in individuals without diabetes.[24] Tear break up

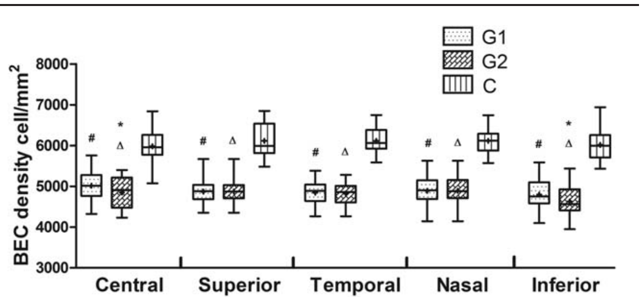

Figure 3. The BEC density in different cornea zones among the type 2 diabetes mellitus and control groups. The "+" is the mean of the data set. BEC=basal epithelial cell, C=control group, G1=type 2 diabetes mellitus group 1, G2=type 2 diabetes mellitus group 2. $^{*}P<.05$ (type 2 diabetes mellitus group 1 compared with type 2 diabetes mellitus group 2), $^{\#}P<.01$ (type 2 diabetes mellitus group 1 compared with the control group), $^{\Delta}P<.01$ (type 2 diabetes mellitus group 2 compared with the control group).

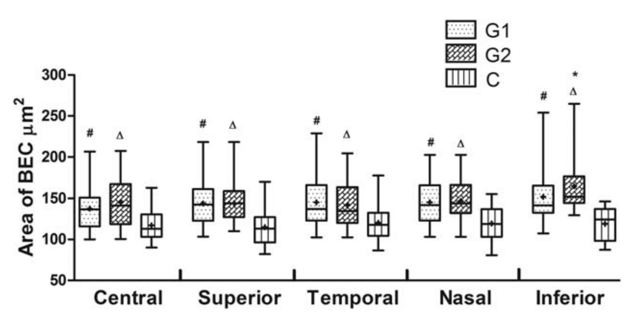

Figure 4. The area of BEC in different cornea zones among the type 2 diabetes mellitus and control groups. The "+" is the mean of the data set. BEC=basal epithelial cell, C=control group, G1=type 2 diabetes mellitus group 1, G2=type 2 diabetes mellitus group 2. $^{*}P<.05$ (type 2 diabetes mellitus group 1 compared with type 2 diabetes mellitus group 2), $^{\#}P<.01$ (type 2 diabetes mellitus group 1 compared with the control group), $^{\Delta}P<.01$ (type 2 diabetes mellitus group 2 compared with the control group).

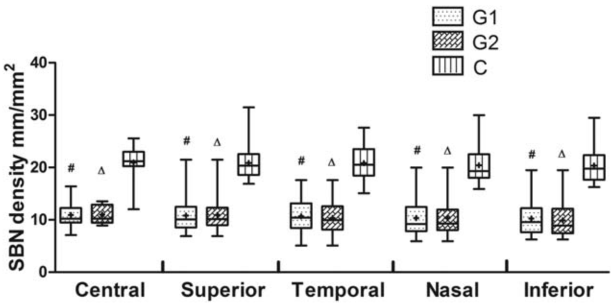

Figure 5. The SBN in different corneal zones among the type 2 diabetes mellitus and control groups. The "+" is the mean of the data set. , C=control group, G1=type 2 diabetes mellitus group 1, G2=type 2 diabetes mellitus group 2, SBN=sub-basal nerve plexus. $^{\#}P<.01$ (type 2 diabetes mellitus group 1 compared with the control group), $^{\Delta}P<.01$ (type 2 diabetes mellitus group 2 compared with the control group).

time has also been shown to be reduced in patients with diabetes.[25] In our research OSDI, Schirmer I test, TMH, and NIKBUTs values were lower in the T2DM group compared with healthy subjects. K5 M has the potential to represent the "true" state of the ocular surface.[26] One study reported good repeatability and reproducibility of TMH and NIKBUTs measurements.[19] Diabetic corneal epitheliopathy has a close relationship with tear film stability and quantity.

Our results were similar to those of Chang et al[11] and Quadrado et al,[8] who reported a lower BEC density in T2DM patients. HbA1c was related to BEC density in T2DM group 1 and group 2; the corneal staining scores of T2DM group 2 in the central and inferior zone were consistent with BEC density decreases. The decrease in BEC density in T2DM patients may result from multifactors. First, corneal innervation can affect corneal epithelial metabolism.[8] Some supply trophic neuropeptides secreted by corneal sensory nerves, which increase corneal epithelial cells mitosis and may regulate epithelial cell differentiation and migration functions.[8] Second, BEC density was increased in T2DM group 2 compared with T2DM group 1, and LC density was also increased in T2DM group 2 compared with T2DM group 1. BEC density in the T2DM groups showed a negative correlation with inferior LC density. This might suggest that LC was participating in the early stages of BEC proliferation and differentiation. Third, an accumulation of AGEs in the cornea, particularly on laminin, may play a causative role in diabetic keratopathy and may partly explain some of the structural changes in the corneal epithelium. Deposition of AGEs changes the molecular structure of the basement membrane and alters the adhesive properties, leading to adhesion complex defects of epithelial cells and delaying the repair of the basement membrane and BEC.[13,27] Thus, BEC density directly evaluated epithelium statement, whereas the central and inferior zones of the cornea were more sensitive to measurement. The area of BEC can also evaluate the epithelium, but BEC density was more precise.

In T2DM patients, Ziegler et al[6] and Zhivov et al[28] reported SBN density to be $19.7\pm7.5\,\text{mm/mm}^2$ and $6.2\pm4.4\,\text{mm/mm}^2$, respectively. In the current article, we also show that SBN density is decreased in T2DM patients, but that the SBN density of the central and peripheral corneal zones showed no difference in T2DM group 1 and group 2. Corneal sensations as measured by esthesiometer and SBN density observed by IVCM were both reduced in T2DM patients. These results are same as those of other studies.[6,28] However, the results of corneal sensation and SBN density were not consistent; IVCM can only reflect the statements of the SBN instead of all corneal nerve receptors.

LCs are mainly located in the central and periphery cornea.[29,30] Most previous studies have[31] reported the location of the LC to be beneath the BEC between the SBN. However, there was no correlation between LC density and glycamic control in our study. It indicates that the increase in LC might be unrelated to hyperglycemia. Tavakoli et al[15] reported corneal nerve damage might be related with an immune-mediated in diabetic patients. Corneal sensation was lower in T2DM group 2 compared with T2DM group 1, and LC density showed the same tendency; this had proved the Tavakoli hypothesis.

In our study, T2DM patients and healthy controls were not found with any visible differences in the morphology of cells. Quadrado et al[8] found the same result.

There are several limitations to our study. All patients were recruited from the cataract clinic of a single department of ophthalmology and are unlikely to be representative of patients seen in a general clinic or of the population. Eyelid and lid margin examinations should be performed. Additionally, the numbers were too small to adjust the corneal epithelium changes in all T2DM patients, and this may lead to errors.

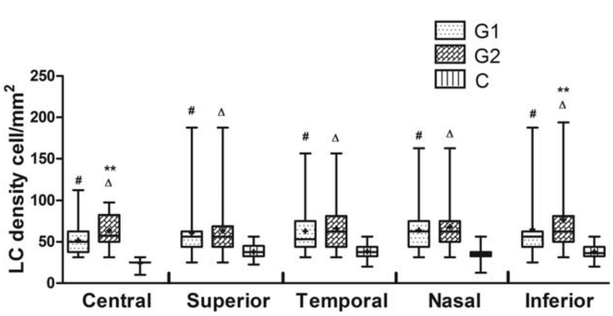

Figure 6. The LC density in different corneal zones among the type 2 diabetes mellitus and control groups. The "+" is the mean of the data set. C=control group, G1=type 2 diabetes mellitus group 1, G2=type 2 diabetes mellitus group 2, LC=Langerhans cell. $^{**}P<.01$ (type 2 diabetes mellitus group 1 compared with type 2 diabetes mellitus group 2), $^{\#}P<.01$ (type 2 diabetes mellitus group 1 compared with the control group), $^{\Delta}P<.01$ (type 2 diabetes mellitus group 2 compared with the control group).

5. Conclusion

In summary, LC-induced immune-mediated contribution to corneal nerve damage and may influence early stage of BEC proliferation and differentiation in T2DM. BEC density was a reliable index for evaluating the early condition of diabetic corneal epitheliopathy. The BEC density of central and inferior corneal zones was more sensitive.

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