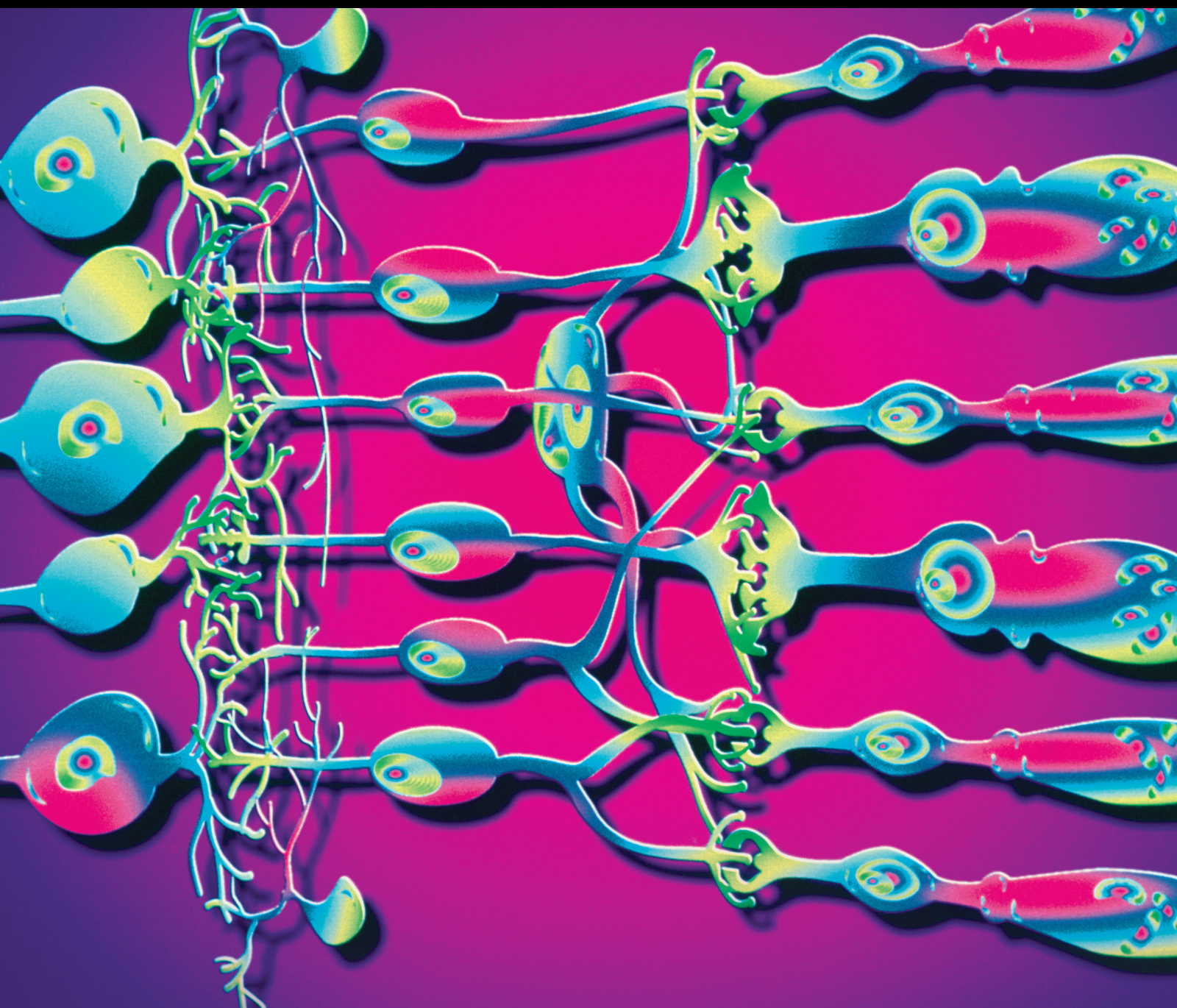
Journal of Ophthalmology

Diabetic Ocular Surface and Anterior Segment Pathology

Lead Guest Editor: Valentin Huerva
Guest Editors: Francisco J. Ascaso and Andrzej Grzybowski



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Contents

Hindawi
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Editorial

Diabetic Ocular Surface and Anterior Segment Pathology

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Ocular diabetes has a great morbidity in the world. Diabetic retinopathy (DR) is a well-known vascular entity, and it may be the most frequent cause of visual loss in these patients. Besides the retinal involvement, diabetes mellitus (DM) may affect the anterior ocular segment, especially the ocular surface. Altered corneal sensory nerves and neurotrophic defects are often found in these patients. Likewise, an associated dry eye can also be present. Cataracts are common in the diabetic population. The risk of diabetic macular edema following phacoemulsification surgery is increased. Intravitreal anti-VEGF agents and corticosteroids are used frequently. Nevertheless, these repetitive therapies could be toxic for the corneal endothelium.

Good glycemic control is essential to avoid the appearance of pathological alterations in the previously mentioned territories. The ocular surface serves to help correct diagnosis and follow-up of the diabetic patients. The measurement of glucose concentration in the tear film is a noninvasive test which can show blood glucose changes throughout the day without needing punctures or blood tests.

Confocal microscopy helps to understand what happens in corneal sensory receptors. Different methods to determine the glycemic levels in the tear film have been investigated. 24-hour contact lens sensor monitoring is really novel and can be useful in the future. On the other hand, corneal sensitivity may be altered in diabetes due to the systemic polyneuropathy present in DM. The alterations of corneal sensitivity can cause a dry eye due to an absence or reduction of the afferent reflex, and tear osmolarity may be increased.

X. Zeng et al. demonstrated that all lacrimal units may be altered during the course of type 2 DM. Parameters such as SPEED score, Schirmer I test, meibography, Ni-BUT, and lipid layer thickness, among others, worsened with the duration of the disease. G. Qin et al. reported observations about Buddleja officinalis Maxim eye drops in an experimental dry eye rabbit model. These eye drops can improve the morphological structure of the lacrimal gland in castrate dry eye rabbits.

Another highlight of the special issue was reported by Y. Xiao et al. about possible variations between type 1 DM duration and biometric parameters in Chinese children. They found no correlation between HbA1c and duration of the type 1 diabetes and the biometric parameters in children.

Structural and biomechanical corneal differences between diabetics type 2 and nondiabetics patients are reported by J. N. Beato et al. No differences were found in corneal hysteresis and corneal resistance factor between both groups.

K. Krysik et al. reported a pachymetric evaluation in diabetic patients with Scheimpflug camera and swept-source optical coherence tomography. They compared pachymetry in diabetics type 2 and nondiabetics individuals.

An update on corneal biomechanics and architecture in DM is reviewed by M. A. del Buey et al. The epithelium, stroma, endothelium, and corneal nerves suffer specific complications during the disease.

Finally, a study by D. S. Lomoriello et al. shows that an early subclinical alteration in subbasal nerve corneal plexus is detected by confocal microscopy in absence of other diabetic complications, including microvascular diabetic complications, diabetic peripheral neuropathy, diabetic autonomic neuropathy, diabetic retinopathy, and microalbuminuria.

In summary, this special issue offers an overview of the alterations of DM in the ocular surface and the anterior ocular segment. It also provides new findings that may be clue for new research in this important field.

Conflicts of Interest

The editors declare no conflicts of interest.

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Valentin Huerva
Francisco J. Ascaso
Andrzej Grzybowski

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Research Article

Early Alterations of Corneal Subbasal Plexus in Uncomplicated Type 1 Diabetes Patients

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Purpose. The purpose of our study is to describe the in vivo corneal confocal microscopy characteristics of subbasal nerve plexus in a highly selected population of patients affected by type 1 diabetes mellitus (T1DM) without any microvascular diabetes complications. *Methods*. We included 19 T1DM patients without diabetic peripheral neuropathy, diabetic autonomic neuropathy, diabetic retinopathy, and microalbuminuria. All patients underwent in vivo corneal confocal microscopy and blood analysis to determine subbasal nerve plexus parameters and their correlation with clinical data. We compared the results with 19 healthy controls. *Results*. The T1DM group showed a significant decrease of the nerve fiber length ($P = 0.032$), the nerve fiber length density ($P = 0.034$), the number of fibers ($P = 0.005$), and the number of branchings ($P = 0.028$), compared to healthy subjects. The nerve fiber length, nerve fiber length density, and number of fibers were directly related to the age at onset of diabetes and inversely to the duration of DM. BMI (body mass index) was highly related to the nerve fiber length ($r = -0.6$, $P = 0.007$), to the nerve fiber length density ($r = -0.6$, $P = 0.007$), and to the number of fibers ($r = -0.587$, $P = 0.008$). No significant correlations were found between the corneal parameters and HbA1c. *Conclusions*. Early subclinical fiber corneal variation could be easily detected using in vivo corneal confocal microscopy, even in type 1 diabetes without any microvascular diabetes complications, including diabetic peripheral neuropathy, diabetic autonomic neuropathy, diabetic retinopathy, and microalbuminuria.

1. Introduction

Diabetic neuropathy (DN) is an important complication of diabetes mellitus (DM). An early diagnosis of DN has an increasing importance in diabetes management, so the literature is focused on identifying predictive factors for the onset of DN [1]. Alterations of morphological and structural parameters of corneal nerves in patients affected by DM have been already described in the literature [2]. Recently, corneal neuropathy has been suggested as a predictive sign of peripheral neuropathy. In particular, structural changes of the subbasal plexus (SBP) have been reported [3].

In vivo corneal confocal microscopy (IVCCM) is a noninvasive, rapid, and repeatable technique used to obtain in vivo images of the corneal structure, from the endothelium to the epithelium. Therefore, it is considered a diagnostically valid examination for the evaluation of systemic neuropathic processes. With IVCCM, a reduction of fiber density and an increase of fiber tortuosity, related to diabetic peripheral neuropathy (DPN) severity, has been observed in corneal subbasal nerve fibers in patients with DPN [3–5]. Moreover, it has been suggested that chronic hyperglycemia could induce a mitochondrial dysfunction, through an increased oxidative metabolism [3, 6]. Accordingly, several studies based on IVCCM exams reported

mitochondria larger in shape and less metabolically active. Those accumulations of mitochondria and glycogen particles appear as local axon enlargement called beadings [7, 8].

The aim of our study is to describe the characteristics of corneal SBP in a highly selected population of subjects affected by type 1 diabetes (T1DM), without microvascular diabetes complications, including DPN, diabetic autonomic neuropathy (DAN), diabetic retinopathy (DR), and microalbuminuria.

2. Materials and Methods

2.1. Study Population. We have screened patients referring to the Unit of Endocrinology, Diabetes and Metabolism, Department of Systems Medicine in S. Giovanni Calibita Fatebenefratelli Hospital, University of Rome Tor Vergata, Rome, Italy, from March 1, 2017, to March 30, 2018. All patients enrolled were aged >18 years, and they were affected by T1DM, according to American Diabetes Association (ADA) criteria [9]. Exclusion criteria were as follows:

(1) Symptomatic peripheral diabetic polyneuropathy even without positive sensory symptoms such as pain, burning, paraesthesia, or prickling

(2) A Michigan Diabetes Neuropathy Instruments [10] total score equal to or greater than 2 points

(3) DAN evaluated by Ewing battery [11]

(4) History of possible confounding diseases (inflammatory diseases, alcohol abuse, vitamin deficiency, malignancy treated with chemotherapy agents, recent history of heart or respiratory failure, chronic liver or renal failure central nervous system diseases, entrapment mononeuropathies, and cervical or lumbosacral radiculopathies)

(5) Microalbuminuria (urinary albumin/creatinine ratio >30 mg/g)

All patients underwent a general medical examination and anthropometric parameters. After an overnight fast, blood and urine samples were obtained for the determination of laboratory measurements. We performed blood tests to measure TGL, CT, HDL, LDL, and creatinine in all diabetes mellitus type 1 patients in order to describe the metabolic characteristics of the population and to rule out the confounding effect of high lipid values or renal failure on SBP parameters. Regarding healthy subjects, we performed an oral glucose tolerance test in order to exclude diabetes and impaired glucose tolerance. We, also, excluded subjects with dyslipidemia, chronic renal failure, and hypertension based on the medical history. A complete ophthalmic examination was carried out in all subjects recruited for the study. Ocular exclusion criteria were the diagnosis of diabetic retinopathy (DR), contact lenses wearing, history of refractive, glaucoma or retinal surgery, ocular medications, with the exception of artificial tears, cataract surgery within the last 6 months, and eye inflammation. All research procedures described in this work adhered to the tenets of the Declaration of Helsinki. All subjects recruited allowed written informed consent after a full explanation of the procedure.

2.2. Laboratory Measurements. Blood and urinary samples were analysed as described in a previous work of our group [12].

HbA1c was quantified by high-performance liquid chromatography (VARIANT 2; BioRad Laboratories, Munich, Germany), with intra- and interassay CV of 0.46–0.77 and 0.69–0.91%, respectively. Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analysed with a colorimetric enzymatic method (CHOD-PAP; Roche Diagnostics). The intraassay CV was 1%, and the interassay CV was 2.7%. The sensitivity of the method was 0.08 mmol/L. Plasma triglycerides were analysed with a colorimetric enzymatic method (GPO-PAP; Roche Diagnostics). The intraassay CV was 1.5%, and the interassay CV was 2.4%. The sensitivity of the method was 0.05 mmol/L. Urinary albumin was determined by the Tina-quant immunoturbidimetric assay (Cobas; Roche Diagnostic, Indianapolis, IN) and urinary creatinine by the enzymatic colorimetric test (Beckmann Coulter, California, USA).

2.3. In Vivo Corneal Confocal Microscopy (IVCCM). IVCCM (Confoscan 4; Nidek Technologies, Gamagori, Japan) was performed bilaterally on the central cornea of all patients at the anterior segment unit of IRCSS Fondazione Bietti, Rome, Italy.

A total of 19 healthy patients matched by sex and age were included as control.

After the application of one drop of topical aneasthetic, 0.4% oxybuprocaine chlorohydrate (Novesina, Novartis Farma, Varese, Italy), a transparent and sterile viscous gel (dexpantenol 5%) was applied to the tip of the lens. This eliminates the optical interfaces with different refractive indices, keeping constant the refractive index, and allows to maintain the desired focal distance. Furthermore, the interposition of the gel allowed a no-contact examination with invasiveness. The *z*-ring was used in all cases. The standard dimension of each image was $340 \times 255\,\mu$m, with an optical section thickness of $5.50\,\mu$m. The overall examination took 2 to 3 minutes. Nobody among patients complained corneal symptoms or visual complications after the examination.

2.4. Corneal Subbasal Nerve Plexus Analysis. The images have been selected from the layer immediately at, or posterior to, the basal epithelial layer and anterior to Bowman's layer. For each patient, the best focused frame of the SBP was chosen.

The analysis of corneal nerve fibers was performed later using CS4 Nerves Tracking Tool CS4 software v1.3.0 and manual edit (Figure 1).

All examinations were obtained by the same experienced operator (DSL), who selected the best focused image

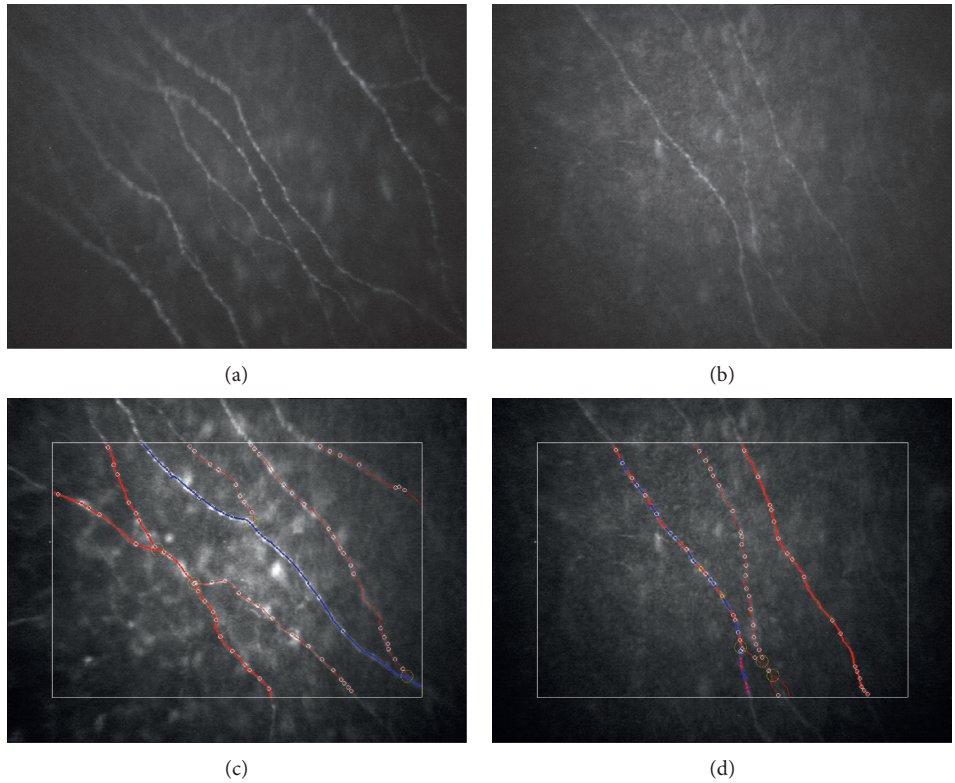

Figure 1: Representative IVCCM images of (a) a healthy control; (b) a T1DM patient with no DPN; (c) corneal nerve fiber image analysis using CS4 Nerves Tracking software of a healthy control; (d) corneal nerve fiber image analysis using CS4 Nerves Tracking software of a T1DM patient.

for each patient. A second, masked, experienced operator (IA) performed the analysis of frames. After automated identification of fibers, two operators (DSL and IA), who were masked to group assignment, reviewed each area and manually corrected any error.

The corneal SBP parameters analysed were 7 and are as follows:

(1) Nerve fiber length, the total length of all fibers and branches/frame (μm/frame)

(2) Nerve fiber length density, the total length of the nerve fibers in $\mu m/mm^2$

(3) Number of fibers, the total number of nerve fibers, including main nerves and branches

(4) Number of branchings, points where nerve branches arise from main nerve

(5) Number of beadings, the total number of well-defined hyperreflective points in all identified main nerves (trunks, long fibers that crossed the borders of the area of analysis in one image)

(6) Beadings density, the total number of nerve beadings divided by the total length of nerve trunks in millimeter (beadings/mm)

(7) Nerve fiber tortuosity using Nidek Nerve index, a unitless measure which represents the degree of twistedness of a curved structure

3. Statistical Analysis

Statistical evaluation was considered using SPSS (IBM SPSS Statistics 25). All results were expressed as the mean-$\pm$standard deviations. The normal data distribution was tested by using the one-sample Kolmogorov–Smirnov test. In order to compare differences in parameters between the diabetic patient group and healthy subjects, the independent-sample t test and the Mann–Whitney test were used as appropriate. To study the relationship between parameters, the Pearson correlation coefficient was computed. In all analyses, $P < 0.05$ was considered to be statistically significant.

4. Results

A total of 19 patients affected by T1DM were included (10 females and 9 males) and compared to a healthy control group of 19 patients (10 females and 9 males).

The clinical and demographic characteristics of the T1DM group are described in Table 1. Both groups were comparable by age (T1DM 37.42 ± 8.99 versus control 40.31 ± 11.15, $P = 0.384$).

All participants underwent corneal SBP analysis. The nerve fiber length (T1DM group (866.45 ± 432.04) versus control group (1186.20 ± 450.02), $P = 0.032$), the nerve fiber length density (T1DM group (9808.96 ± 4808.96) versus

Table 1: Demographic, metabolic, and anthropometric characteristics of the study population.

	T1DM ($n = 19$)
Age (mean ± SD) (years)	37.42 ± 8.99
Sex (male/female)	10/9
Age at onset of DM (years)	24.94 ± 10.18
Duration of DM (years)	12.47 ± 8.29
BMI (Kg/m^2)	25.03 ± 4.63
HBA1c (%)	7.62 ± 0.84
TG (mg/dl)	77 ± 30.45
TC (mg/dl)	180.95 ± 26.94
HDL-C (mg/dl)	66.37 ± 15.96
LDL-C (mg/dl)	99.71 ± 24.97
Creatinine (mg/dl)	0.77 ± 0.12
Microalbuminuria/creatininuria (mg/g)	8.62 ± 8.13

BMI, body mass index; HbA1c, glycosylated hemoglobin; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol.

control group (13357.22 ± 5056.19), $P = 0.034$), the number of fibers (T1DM group (4.68 ± 2.11) versus control group (7.16 ± 2.87), $P = 0.005$), and the number of branchings (T1DM group (1.89 ± 1.56) versus control group (3.26 ± 1.99), $P = 0.028$) were significantly lower in the T1DM group compared to those in the healthy subjects, while the number and density of beadings and nerve fiber tortuosity did not differ between the two groups (Table 2). In T1DM group, the age at onset of diabetes was directly related to the nerve fiber length ($r = 0.535$, $P = 0.018$), the nerve fiber length density ($r = 0.524$, $P = 0.02$), and the number of fibers ($r = 0.444$, $P = 0.05$). The same SBP parameters were inversely related to the duration of DM (nerve fiber length: $r = -0.657$, $P = 0.002$; nerve fiber length density: $r = -0.666$, $P = 0.002$; number of fibers: $r = -0.610$, $P = 0.006$). None of nerve fiber parameters was related to the age of the patients at the time of the examinations (Table 3). No significant correlations were found between the corneal parameters and HbA1c. BMI of the T1DM group was highly related to the nerve fiber length ($r = -0.6$, $P = 0.007$), the nerve fiber length density ($r = -0.6$, $P = 0.007$), and the number of fibers ($r = -0.587$, $P = 0.008$) (Table 4).

We also compared T1DM and healthy groups, according to the sex. Females were comparable by age, and all corneal SBP parameters did not statistically differ between healthy and T1DM subjects. Analysing the SBP data in males, instead, the nerve fiber length (T1DM group (697.96 ± 101.48) versus control group (1415.42 ± 132.39), $P = 0.001$), the nerve fiber length density (T1DM group (7978.80 ± 1158.24) versus control group (15932.77 ± 1489.73), $P = 0.001$), the number of fibers (T1DM group (4.11 ± 0.75) versus control group (8.78 ± 0.83), $P = 0.001$), the number of branchings (T1DM group (1.78 ± 0.57) versus control group (3.89 ± 0.73), $P = 0.026$), and the number of beadings (T1DM group (16.67 ± 1.21) versus control group (21.11 ± 1.36), $P = 0.029$) were found to be significantly lower in T1DM males, compared to healthy males. Age, beading density, and corneal nerve tortuosity did not differ between diseased and healthy males (Table 5). Dividing the patients within the diabetic group by sex, we observed that the nerve fiber length (male T1DM (697.96 ± 101.48) versus female T1DM (1018.09 ± 153.95); $P = 0.05$) and the nerve fiber length density (male T1DM (7978.80 ± 1158.24) versus female T1DM (11456.11 ± 1732.28); $P = 0.05$) were statistically lower in males compared to females. However, this difference was caused by the lower age at onset of diabetes in males compared to females (male T1DM (19.55 ± 2.52) versus female T1DM (29.8 ± 3.19), $P = 0.045$), as we found repeating the statistical analysis on studentized residuals after correction for age at onset (fiber length $P = 0.106$; fiber length $P = 0.097$). BMI and blood parameters did not differ between female and male in the T1DM group (Table 6).

In healthy controls, none of corneal SBP parameters was sex-related. On the other hand, in T1DM males, we found an inverse relation between the duration of DM and two corneal parameters, the nerve fiber length ($r = -0.690$, $P = 0.04$), and the nerve fiber length density ($r = -0.718$, $P = 0.029$). BMI had an indirect correlation with the nerve fiber length ($r = -0.856$, $P = 0.003$), the nerve fiber length density ($r = -0.855$, $P = 0.002$), and the number of fibers ($r = -0.774$, $P = 0.014$). In the female subgroup of T1DM, we did not found any correlation with clinical age, age at onset, and DM duration (Table 7).

5. Discussion

The utility of IVCCM to define the corneal SBP in diabetic patients with or without DPN has already been reported [4, 13]. The concept of corneal neuropathy with corneal fiber damages in diabetes was introduced by the Malik group [14], describing a significant reduction of corneal nerve fiber density, length, and branch density in diabetic patients with DPN compared to healthy controls. The aim of our study was to investigate the characteristics of corneal SBP in adult T1DM without DPN and any other ocular signs or symptoms, including DR, and to correlate them with clinical and anthropomorphic data. We excluded patients affected by DR from our population, because a relationship between the decrease of number of corneal fiber nerve and severity of DR has already been described [15, 16]. Furthermore, diabetic neuropathy and retinal neurodegeneration could anticipate a clinical evident retinopathy [12, 16].

In our study, we observed that the nerve fibers length, the nerve fibers density, the number of fibers, and the number of branchings were statistically lower in patients affected by T1DM compared to nondiabetic controls. These differences were observed in our group of highly selected diabetic subjects without any microvascular complications. Moreover, the good glycemic and metabolic control and the lack of comorbidities allowed to carry out these evaluations without confounding factors.

Our results were aligned to other studies in the literature. Ishibashi's group described that, in corneal SBP, the nerve fiber length, the nerve fiber density, and the branch density were lower in patients affected by T2DM without DPN, compared with those of healthy controls [6].

Edwards et al., comparing diabetic patients with and without DPN, demonstrated the nerve fiber length and the nerve branch density reduction in patients affected by

Table 2: Summary of corneal nerves morphological parameters in study population (T1DM versus healthy control).

Corneal nerves parameters	Healthy control ($n = 19$)	T1DM ($n = 19$)	P value
Nerve fiber length (μm/frame)	1186.20 ± 450.02	866.45 ± 432.04	0.032*
Nerve fiber length density (μm/mm^2)	13357.22 ± 5056.19	9808.96 ± 4853.05	0.034*
Number of fibers (no./mm^2)	7.16 ± 2.87	4.68 ± 2.11	0.005*
Number of branchings (no.)	3.26 ± 1.99	1.89 ± 1.56	0.028*
Number of beadings (no.)	19.68 ± 3.54	16.21 ± 5.12	0.056
Beadings density (no./mm)	71.37 ± 10.30	65.62 ± 21.85	0.306
Nerve fiber tortuosity	6.02 ± 2.66	5.61 ± 1.75	0.737

Table 3: Correlation r ($P < 0.05$) of corneal parameters in the T1DM group with clinical and metabolic data.

Corneal nerves parameters	Age	Age at onset	Duration of DM	BMI (kg/m^2)	HBA1c (%)
Nerve fiber length (μm/frame)	0.01	1.535 (0.018)*	−0.657 (0.002)*	−0.6 (0.007)*	0.068
Nerve fiber length density (μm/mm^2)	−0.021	1.524 (0.021)*	−0.666 (0.002)*	−0.6 (0.007)*	0.074
Number of fibers (no./mm^2)	−0.060	1.444 (0.05)*	−0.610 (0.006)*	−0.587 (0.008)*	−0.062
Number of branchings (no.)	−0.013	0.237	−0.305	−0.293	−0.210
Number of beadings (no.)	−0.228	0.088	−0.355	0.044	0.406
Beadings density (no./mm)	−0.210	−0.124	−0.075	0.404	0.292
Nerve fiber tortuosity	0.344	0.258	0.056	−0.202	−0.188

No significant correlations were found between the corneal parameters and HbA1c. BMI of the T1DM group was highly related to the nerve fiber length ($r = -0.6$, $P = 0.007$), the nerve fiber length density ($r = -0.6$, $P = 0.007$), and the number of fibers ($r = -0.587$, $P = 0.008$) (Table 4).

Table 4: Comparison between the healthy control and T1DM patients divided by sex.

Female	Healthy control ($n = 10$)	T1DM ($n = 10$)	P value
Age (mean ± SD)	38 ± 4.3	39.4 ± 2.64	0.785
Nerve fiber length (μm/frame)	979.44 ± 128.28	1018.09 ± 153.95	0.849
Nerve fiber length density (μm/mm^2)	11039.22 ± 1441.25	11456.11 ± 1732.28	0.855
Number of fibers (no./mm^2)	5.70 ± 0.77	5.20 ± 0.61	0.619
Number of branchings (no.)	2.70 ± 0.54	2.00 ± 0.47	0.341
Number of beadings (no.)	18.4 ± 0.81	15.80 ± 2	0.244
Beadings density (no./mm)	68.87 ± 2.32	61.44 ± 7.3	0.345
Nerve nerve fiber tortuosity	5.16 ± 0.53	5.88 ± 0.50	0.336
Male	Healthy control ($n = 9$)	T1DM ($n = 9$)	P value
Age (mean ± SD)	42.89 ± 2.52	35.22 ± 3.21	0.079
Nerve fiber length (μm/frame)	1415.42 ± 132.39	697.96 ± 101.48	0.001*
Nerve fiber length density (μm/mm^2)	15932.77 ± 1489.73	7978.80 ± 1158.24	0.001*
Number of fibers (no./mm^2)	8.78 ± 0.83	4.11 ± 0.75	0.001*
Number of branchings (no.)	3.89 ± 0.73	1.78 ± 0.57	0.026*
Number of beadings (no.)	21.11 ± 1.36	16.67 ± 1.21	0.029*
Beadings density (no./mm)	74.16 ± 4.23	70.27 ± 6.91	0.637
Nerve fiber tortuosity	6.98 ± 0.09	5.30 ± 0.65	0.070

DPN [17]. In particular, corneal nerve fiber length could be considered predictive of DPN [18, 19].

Other studies already described SBP alteration in T1DM without DR compared to that in healthy controls [15, 16]. They reported a reduction of the nerve fiber density and the nerve fiber length. Our study, compared to the previous study of Petropoulos and Burdova, examined also the metabolic activity of the fiber, expressed as number and density of beadings [8].

Regarding beadings, we did not detect any difference between our diabetic patients and controls. Our results about beadings were in contrast with the studies of Ishibashi's group, where they described a lower beading frequency in T1DM without DPN, compared to controls and a consequently reduction of the number of beadings and alteration of their size [6, 20]. They hypothesized that these alterations could be caused by changes in the distribution of mitochondria, which became detectable before the onset of DPN. We supposed that in our population of adult T1DM without DPN, the good glycemic and metabolic control could justify the absence of this difference in beading parameters of diabetics, compared to controls. However, this difference could be also due to the different methodologies. Indeed, both Ishibashi and Tavakoly used a manual method to count the beadings and numbered the beadings for 0.1 mm of fiber, while in our study, we performed an automatic count and revised manually [2, 21]. Moreover, Ishibashi did not specify whether T1DM patients were affected by DR, which could be associated with an early degeneration of corneal fibers, with a possible variation of beadings too.

In our population, the tortuosity index did not differ from controls as well. This is opposite to the previous

Table 5: Comparison between male and female in the T1DM population.

	Male ($n = 9$)	Female ($n = 10$)	P value
Age (mean ± SD)	35.22 ± 3.21	39.40 ± 2.64	0.326
Age at onset of DM	19.55 ± 2.52	29.8 ± 3.19	0.045*
Duration of DM (years)	15.67 ± 3.09	9.60 ± 2.05	0.113
BMI (kg/m^2)	26.81 ± 1.75	23.42 ± 1.11	0.102
HBA1c (%)	7.59 ± 0.22	7.65 ± 0.32	0.880
TG (mg/dl)	90.55 ± 12.23	64.8 ± 5.56	0.063
CT (mg/dl)	170.66 ± 6.19	190.20 ± 9.72	0.117
HDL (mg/dl)	60.78 ± 5.28	71.40 ± 4.76	0.153
LDL (mg/dl)	91.78 ± 5.13	106.86 ± 9.57	0.197
Creatinine (mg/dl)	0.78 ± 0.04	0.76 ± 0.04	0.777
Microalbuminuria/creatininuria (mg/g)	7.6 ± 2.17	9.54 ± 3.04	0.617
Nerve fiber length (μm/frame)	697.96 ± 101.48	1018.09 ± 153.95	0.05*
Nerve fiber length density (μm/mm^2)	7978.80 ± 1158.24	11456.11 ± 1732.28	0.05*
Number of fibers (no./mm^2)	4.11 ± 0.75	5.20 ± 0.61	0.273
Number of branchings (no.)	1.78 ± 0.57	2.00 ± 0.47	0.735
Number of beadings (no.)	16.67 ± 1.21	15.80 ± 2	0.724
Beadings density (no./mm)	70.27 ± 6.91	61.44 ± 7.3	0.744
Nerve fiber tortuosity	5.30 ± 0.65	5.88 ± 0.50	0.253

Table 6: Correlation r ($P < 0.05$) of corneal parameters in the T1DM subgroup divided by sex and clinical data.

Corneal nerve parameters	Age		Age at onset		Duration of DM	
	Male	Female	Male	Female	Male	Female
Nerve fiber length (μm/frame)	−0.347	0.051	0.403	0.441	−0.690 (0.040)*	−0.620
Nerve fiber length density (μm/mm^2)	−0.398	0.051	0.372	0.441	−0.718 (0.029)*	−0.620
Number of fibers (no./mm^2)	−0.391	0.180	0.267	0.470	−0.625	−0.498
Number of branchings (no.)	−0.578	0.615	−0.105	0.503	−0.517	0.011
Number of beadings (no.)	−0.215	−0.233	0.121	0.170	−0.323	−0.564
Beadings density (no./mm)	−0.243	−0.103	−0.360	0.182	−0.040	−0.416
Nerve fiber tortuosity	0.605	−0.045	0.508	−0.038	0.216	0.001

Table 7: Correlation r ($P < 0.05$) of corneal parameters in the T1DM subgroup divided by sex and metabolic data.

Corneal nerve parameters	BMI		HBA1c	
	Male	Female	Male	Female
Nerve fiber length (μm/frame)	−0.856 (0.003)*	−0.358	−0.183	0.145
Nerve fiber length density (μm/mm^2)	−0.855 (0.003)*	−0.358	−0.155	0.145
Number of fibers (no./mm^2)	−0.774 (0.014)*	−0.198	−0.426	0.159
Number of branchings (no.)	−0.579	0.154	−0.355	−0.133
Number of beadings (no.)	−0.094	0.097	0.040	0.535
Beadings density (no./mm)	0.452	0.276	−0.001	0.471
Nerve fiber tortuosity	−0.051	−0.316	0.201	−0.496

reported data by Kallinikos et al., who found that the fiber tortuosity index seemed to be related to the degenerative mechanism and the regenerative response of nerve fibers in diabetes [22]. Moreover, the difference in this result could be justified by the fact that our population of diabetics did not have DPN, unlike the Kallinikos study group.

In our study, age at onset was directly associated to the nerve fibers length, the nerve fibers density, and the number of fibers. These SBP parameters were instead inversely related to DM duration. These results were in agreement with the current literature [16, 22]. None of the corneal parameters were related to the age of patients at the time of exams. It has been already described that fiber nerve number and density and also the number of beadings did not statistically reduce with age in young adults [16, 23, 24].

As for the clinical data, the nerve fibers length, the nerve fibers density, and the number of fibers were inversely related to BMI. In our study, we also confirmed the lack of correlation between HbA1c and corneal parameters, as already reported in the literature [25, 26].

We had divided our study population according to the sex. In female, there was no difference between the SBP parameters of T1DM and those of healthy controls. On the other hand, T1DM males showed a reduction in corneal nerve fiber length, corneal nerve fiber length density, the number of fibers, the number of branchings, and the number of beadings compared to healthy males. Therefore, we

studied the differences in corneal nerves parameters in T1DM divided by sex and we found that fiber length and fiber length density were lower in diseased males than in females ones. However, analysing our data, it was found that these differences were not sex-related, but rather related to the age of onset of diabetes, which was earlier in males than in females. These results underline the importance of the age of onset on corneal parameters alterations.

The main limitation of the assessment of our results was the small number of the examined patients, slightly due to the strict inclusion criteria we assumed. Indeed, we excluded all adult diabetics with any microvascular complication, including DPN, DAN, DR, and microalbuminuria. Nevertheless, our study demonstrates the presence of corneal SBP alterations even in a highly selected subgroup of diabetics. A longitudinal study carrying out an IVCCM in a larger group of diabetic patients before the onset of disease-related complications would be further investigated.

6. Conclusions

An alteration of corneal subbasal plexus is already present in subjects affected by T1DM highly selected, without microvascular complications and comorbidities, and in good glycemic and metabolic controls. IVCCM confirms to be a noninvasive and helpful tool in the diagnosis of early diabetic alterations.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Research Article

Intraocular Pressure and Anterior Segment Morphometry Changes after Uneventful Phacoemulsification in Type 2 Diabetic and Nondiabetic Patients

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Purpose. To compare intraocular pressure (IOP) and anterior segment (AS) morphometry changes after uneventful phacoemulsification between nonglaucomatous eyes with open-angles from patients with and without type 2 diabetes mellitus (DM) and determine which factors may predict greater IOP-lowering effect. *Methods*. Forty-five diabetic (45 eyes) and 44 (44 eyes) age- and sex-matched non-DM patients with age-related cataract were enrolled in this prospective observational study. Goldmann applanation tonometry and AS Scheimpflug tomography (Pentacam® HR) were performed preoperatively and at 1- and 6-month follow-up. Linear regression analysis was performed to evaluate the clinical variables related to postoperative IOP changes at 6 months. *Results*. There was a significant postoperative IOP reduction 6 months after surgery ($p < 0.001$) by an average of 2.9 ± 2.9 mmHg (15.5%) and 2.4 ± 2.8 mmHg (13.0%) in the DM group and non-DM groups ($p = 0.410$), respectively. All AS parameters (anterior chamber depth, volume, and angle) increased significantly postoperatively ($p < 0.001$). Multivariate linear regression analysis showed that higher preoperative IOP was significantly associated with IOP reduction at 6-month follow-up ($p < 0.05$). *Conclusion*. Nonglaucomatous eyes with open-angles from both type 2 diabetic and nondiabetic patients experienced similar AS changes and IOP reductions following uneventful phacoemulsification, and this IOP-lowering effect was strongly correlated with preoperative IOP.

1. Introduction

Over the last two decades, several studies have consistently shown a significant and sustained intraocular pressure (IOP) decrease after uneventful phacoemulsification cataract surgery and posterior chamber intraocular lens (IOL) implantation in eyes either with or without ocular hypertension or glaucoma [1]. Although the pressure lowering mechanisms remain under debate, an improved aqueous access to the trabecular meshwork undoubtedly plays an important role, especially in eyes with partially or completely closed angles [2–5].

Anterior segment (AS) imaging has become progressively attractive with the advent of new high-resolution noncontact technologies, such as Scheimpflug-based systems (e.g., Pentacam® HR). These devices enable objective evaluation and quantification of several AS parameters (anterior chamber depth (ACD), volume (ACV), and angle (ACA)) [6], which have been studied as predictive markers of IOP reductions following cataract surgery [7–11].

The relationship between AS biometric changes and elevated plasma glucose concentrations in diabetes mellitus (DM) has been studied in the past. Most importantly, diabetic patients have been found to have thicker lenses and

shallower anterior chambers [12, 13]. Furthermore, in some population-based studies, diabetic subjects had statistically significant higher IOP readings compared to nondiabetics [14]. Given the inverse correlation between preoperative IOP and ACD with postoperative IOP changes after cataract surgery [7–11], we hypothesized that diabetic patients could benefit from greater IOP reductions after phacoemulsification when compared to nondiabetics. However, the increased resistance to aqueous humor outflow caused by the hyperglycemia-induced overexpression of fibronectin in the trabecular meshwork could limit this hypotensive effect [15]. To the best of our knowledge, no prospective study specifically addressed the IOP-lowering effect of cataract surgery in diabetic subjects.

This study was designed to assess the IOP and AS biometric changes that occur following uneventful phacoemulsification in nonglaucomatous eyes with open-angles from nondiabetic and type 2 diabetic patients. In addition, it aimed to determine which factors may predict greater IOP reduction after surgery.

2. Methods

2.1. Participants. In this prospective observational study, type 2 diabetic patients with different stages of diabetic retinopahty (DR) and controls, aged 50 or older, were consecutively recruited from the Cataract and Refractive Surgery Unit of the Ophthalmology Department of Centro Hospitalar Universitário São João between September 2015 and March 2016. Informed consent was obtained from each participant before inclusion in the study. The study protocol was approved by the local Ethics Committee of Health and followed the tenets of the Declaration of Helsinki.

Full inclusion criteria are described elsewhere [16]. The exclusion criteria included prior eye surgery or trauma; any eye corneal, retinal or optic nerve pathology except DR; mature cataracts (brown/white) [17]; Goldmann applanation tonometry (IOP-GAT) > 25 mmHg; preoperative ACA in Scheimpflug tomography <20°; pseudoexfoliation syndrome; and current treatment with any form of steroids. Diabetic patients were excluded from the analysis if they had severe nonproliferative diabetic retinopathy (NPDR), proliferative DR (PDR), or diabetic macular edema (DME). No cases of intraoperative complications or use of adjunctive procedures (e.g., adjuvant intravitreal treatment with anti-VEGF or steroids) were included [16].

2.2. Sample Size Calculation. For a type I error of 0.05 and type II error of 0.20 (80% power), considering a mean difference of absolute IOP change ≥ 1.5 mmHg to be significant between the 2 groups and assuming the standard deviation (SD) for non-DM group of 2.5 mmHg, the minimal required sample size would be 44 subjects in each group [11, 17].

2.3. Study Protocol

2.3.1. Preoperative Assessment. All patients underwent preoperative evaluation, within 2 weeks prior to cataract surgery, including general anamnesis and comprehensive ophthalmologic examination (visual acuity testing, refraction, slit-lamp examination, intraocular pressure measurement and indirect ophthalmoscopy).

For ocular biometry, the IOL Master® 500 (software version 7.7) was used. Anterior segment morphometry was evaluated using Pentacam® HR (software version 1.20r87). Measurements were repeated as necessary until high-quality images were obtained. All measurements were performed by an experienced operator (JB) under standard dim light conditions, without cyclopegia, and the patients were told to blink immediately before each examination [16].

Intraocular pressure was averaged from the two measurements performed using Goldmann applanation tonometry. If the two IOP values differed by more than 2 mmHg, then a third measurement was made and the median value was the one considered. The type of cataract (cortical, nuclear, and posterior subcapsular) and nucleus opacity grade (1 (mild) to 4 (white/brown) severity grading system) were classified after pupillary dilatation. The grade of DR was assessed in all diabetic patients using 7 standard ETDRS fundus photographs [18].

At the end of the baseline visit, an experienced nurse recorded vital signs and collected blood samples, by venous puncture, for serum HbA1c analysis [16].

2.3.2. Surgical Technique. All cataract surgeries were performed under topical anesthesia by experienced surgeons. The subjects underwent standard coaxial 2.75 mm clear cornea phacoemulsification technique (Model Infiniti; Alcon Laboratories, Inc., Fort Worth, TX, USA) with in-the-bag 1-piece acrylic posterior chamber IOL (Acrysof® SA60AT (Alcon Laboratories, Inc., Fort Worth, TX, USA) or Akreos® Adapt lens (Baush & Lomb, Inc., Rochester, NY, USA)) implantation. The ophthalmic viscoelastic device used in all patients was Provisc® (sodium hyaluronate 10%; Alcon Laboratories, Inc.).

The same postoperative medication was prescribed to all the patients, and it consisted of 1 mg/ml dexamethasone, 0.3 mg/ml flurbiprofen, and 5 mg/ml levofloxacin eye drops, five times daily 1 week and then tapered gradually over 3 weeks.

2.3.3. Postoperative Assessment. Patients were evaluated at 1 and 6 months postoperatively using a similar protocol to the baseline visit, with the exception of ocular biometry. Each subject was reexamined at the same time of the baseline visit.

2.4. Devices

2.4.1. IOLMaster® 500 (Carl Zeiss Meditec, Jena, Germany). The IOLMaster® 500 is a partial coherence interferometer used for ocular biometry. It automatically measures the anterior corneal keratometry and the axial length, which are fundamental for IOL power calculation and implantation, and have shown a high intra- and interobserver reproducibility [19].

2.4.2. Pentacam® HR (Oculus, Wetzlar, Germany). The Pentacam uses a single 180-degree rotating Scheimpflug camera and a monochromatic blue slit-light source (475 nm) combined with a static camera (for the correction of any eye movement) to generate a three-dimensional high-resolution (HR) image of the anterior segment. The software enables accurate and reproducible automatic evaluation of central corneal thickness (CCT, measured at corneal apex), ACD (from endothelium to anterior surface of lens), ACV (over a diameter of 10 mm centered on the corneal apex), and ACA (the smallest angle in the Scheimpflug images taken in the horizontal section) in phakic eyes [6].

In pseudophakic eyes, anterior IOL surface may occasionally be mistaken with the iris or the IOL-related light reflex; for that reason, postoperative ACD was manually measured from the central corneal endothelium apex to the anterior IOL surface by the same investigator (DR) after adjusting the contrast of the Scheimpflug image [8, 20]. The Scheimpflug image selected for measurement was the one that provided visualization of the whole IOL optic. The value was averaged after 3 consecutive measurements.

2.5. Data and Statistical Analyses. Intraoperative parameters recorded included cumulative dissipated energy (CDE), which represents the amount of ultrasound energy delivered to the eye during the surgery. To determine whether preoperative IOP had an effect on the postoperative IOP change, patients were stratified into five subgroups based on preoperative IOP: 10–14, 15-16, 17-18, 19-20, and 21–25 mmHg [21–23]. Diabetic subjects were also classified into subgroups according to DM duration (<10 and ≥10 years) and HbA1c levels (<7.0 and ≥7.0%). The predictive value of previously described indices for IOP reduction after cataract surgery was investigated: pressure to depth (PD) ratio (preoperative IOP/preoperative ACD) [7]; pressure to volume (PV) ratio (preoperative IOP/preoperative ACV); and pressure to angle (PA) ratio (preoperative IOP/preoperative ACA) [8].

Statistical analysis was performed using the SPSS statistical software (version 21.0 for Mac OS; SPSS Inc., Chicago, IL, USA). In the present study, only the scheduled eye of each patient undergoing monocular cataract surgery was used for statistical analyses. Normality was assessed using distribution plots and Kolmogorov–Smirnov tests. All comparisons between the DM and non-DM groups, as well as between pre- and postoperative periods, were performed with parametric or nonparametric tests, accordingly to the normality of data. Chi-squared or Fisher's exact tests were performed for categorical variables comparison. Linear regression analysis was performed to identify the potential demographical (age and gender), clinical (DM duration and HbA1c levels), ocular (preoperative AL, CCT, ACD, ACV, and ACA), and intraoperative (cataract grade, CDE, and IOL type) variables associated with postoperative IOP changes. Statistical significance for all the analyses was set at a p value less than 0.05.

STROBE guidelines were followed for manuscript elaboration [24].

3. Results

Forty-five diabetic patients and 44 nondiabetic controls were enrolled in the study. The DM and non-DM groups were comparable with regard to their demographic and clinical characteristics, except that HbA1c levels were higher ($p < 0.001$, Mann–Whitney test) and mean cataract grade was lower ($p = 0.032$, Mann–Whitney test) in the DM group (Table 1). In the DM group, a longer duration of DM was significantly associated with higher HbA1c levels ($p = 0.008$, chi^2 test).

3.1. Intraocular Pressure Comparisons. Mean preoperative IOP was 17.8 ± 3.1 mmHg and 16.9 ± 2.9 mmHg in DM and non-DM groups, respectively ($p = 0.188$). IOP was observed to be significantly lower than preoperative value at both 1 and 6 months of follow-up in both groups ($p < 0.001$, paired t-test). There were no statistically significant differences in IOP variation between groups (Table 2).

Of the 89 eyes, 73 eyes (82%) demonstrated IOP reduction (mean decrease −3.6 ± 2.1 mmHg), 5 eyes (6%) experienced no change in IOP, and 11 eyes (12%) experienced IOP increase (mean increase +2.4 ± 1.3 mmHg). The mean baseline IOP of eyes that demonstrated IOP reduction (18.1 ± 2.7 mmHg; 95% CI, 17.4–18.7 mmHg) was significantly higher than those that demonstrated IOP elevation (14.2 ± 1.5 mmHg; 95% CI, 13.1–15.2 mmHg; $p < 0.001$, independent samples t-test). No group differences were observed with regard to the probability of either an increased or decreased IOP 6 months after surgery ($p = 0.767$, Fisher's exact test).

A higher IOP at baseline was associated with greater IOP reduction 6 months after surgery in both DM and non-DM groups (Pearson's correlation coefficient 0.551; $p < 0.001$ vs. 0.462; $p < 0.002$, respectively) (Figure 1). The largest decrease in postoperative IOP occurred in the subgroup with the highest preoperative IOP (21–25 mmHg: −4.8 ± 2.7 in DM group ($n = 9$) and −7.0 ± 1.4 in non-DM group ($n = 4$)); while in the group with the lowest preoperative IOP (10–14 mmHg), the postoperative IOP remained essentially unchanged (−0.4 ± 3.2 in DM group ($n = 9$) and +0.1 ± 1.5 in non-DM group ($n = 7$)). There was no statistically significant difference between IOP subgroups regarding AS changes (Table 3).

There were no statistically significant differences between subgroups of DM duration or HbA1c levels in the DM subjects.

3.2. Scheimpflug Tomography Comparisons

3.2.1. Central Corneal Thickness (CCT) Comparisons. There were no statistically significant differences between groups for the CCT measurements preoperatively, at 1- and 6-month follow-up (Table 2). The mean postoperative CCT at 1 and 6 months did not change significantly from the mean preoperative level in both DM and non-DM groups (paired t-test; $p > 0.05$).

3.2.2. Anterior Chamber Depth (ACD) Comparisons. Mean preoperative ACD was 2.6 ± 0.4 mm and 2.7 ± 0.4 mm in DM and non-DM groups, respectively ($p = 0.135$). ACD

Table 1: Demographic and clinical characteristics of the study population.

	DM group ($n = 45$)	Non-DM group ($n = 44$)	p
Age (y)	72.7 ± 5.7	70.6 ± 6.3	0.106[1]
Female (n)	28 (63%)	27 (61%)	0.934[3]
Right eyes (n)	22 (49%)	29 (66%)	0.105[3]
BMI (kg/m^2)	28.2 ± 3.9	27.9 ± 5.2	0.763[1]
Smoking history (n)	10 (22%)	17 (39%)	0.092[3]
HbA1c levels (%)	6.8 ± 1.0	5.5 ± 0.4	<0.001*[2]
Duration of diabetes (y)	9.1 ± 8.0	n/a	n/a
DR stage (n)			
No apparent DR	39 (87%)	n/a	n/a
Mild to moderate NPDR	6 (13%)		
Oral antidiabetic agents (n)	43 (96%)	n/a	n/a
Insulin treatment (n)	7 (16%)	n/a	n/a
Axial length (mm, preoperatively)	22.9 ± 0.7	23.0 ± 0.8	0.8551
Intraoperative data			
Cataract grade	1.6 ± 0.6	1.9 ± 0.6	0.032*[2]
CDE	9.3 ± 7.1	9.3 ± 6.4	0.997[1]
IOL power	22.1 ± 1.6	22.2 ± 1.8	0.687[2]
Acrysof®/Akreos®	37/8	38/6	0.592[3]

Data were derived from independent samples t-test[1], Mann–Whitney test[2], and chi-square[3] test. Continuous variables are reported as mean ± standard deviation. *$p < 0.05$, statistical significance. BMI, body mass index; CDE, cumulative dissipated energy; DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, nonproliferative DR; IOL, intraocular lens; mm, millimeters; n/a, not applicable; y, years.

Table 2: Pre- and postoperative measurements in the DM and non-DM groups.

	DM group ($n = 45$)	Non-DM group ($n = 44$)	p
CCT (μm)			
Preoperatively	559.4 ± 37.7	558.3 ± 29.2	0.885[1]
1 mo	562.5 ± 35.2	559.6 ± 28.5	0.671[1]
6 mo	554.1 ± 32.1	565.2 ± 31.7	0.107[1]
IOP-GAT (mmHg)			
Preoperatively	17.8 ± 3.1	16.9 ± 2.9	0.188[1]
Δ1 mo	−1.7 ± 2.9	−2.2 ± 2.5	0.347[1]
Δ6 mo	−2.9 ± 2.9	−2.4 ± 2.8	0.410[1]
ACD (mm)			
Preoperatively	2.6 ± 0.4	2.7 ± 0.4	0.135[1]
Δ1 mo	+1.3 ± 0.3	+1.3 ± 0.3	0.675[1]
Δ6 mo	+1.4 ± 0.3	+1.3 ± 0.3	0.438[1]
ACV (mm^3)			
Preoperatively	126.4 ± 33.3	138.0 ± 35.4	0.116[1]
Δ1 mo	+50.1 ± 22.65	+48.2 ± 22.1	0.696[1]
Δ6 mo	+52.6 ± 23.4	+49.3 ± 23.6	0.501[1]
ACA (degree)			
Preoperatively	30.2 ± 5.5	33.0 ± 5.9	0.022*[1]
Δ1 mo	+13.5 ± 4.9	+12.5 ± 5.6	0.356[1]
Δ6 mo	+14.2 ± 5.1	+12.8 ± 6.1	0.231[1]

Data were derived from independent samples t-test[1], Mann–Whitney test[2], and chi-squared test[3]. Continuous variables are reported as mean-± standard deviation. *$p < 0.05$, statistical significance. [a]CCT measured by Pentacam at corneal vertex. ACA, anterior chamber angle; ACD, anterior chamber depth; ACV, anterior chamber volume; CCT, central corneal thickness; GAT, Goldmann applanation tonometry; K, keratometry; IOP, intraocular pressure; mo, month; PD, pressure-to-depth ratio; Δ, variation.

was observed to be significantly greater than preoperative value at 1 and 6 months of follow-up in both groups ($p < 0.001$, paired t-test). No group differences were observed with regard to ACD variations at 1 and 6 months after surgery (Table 2).

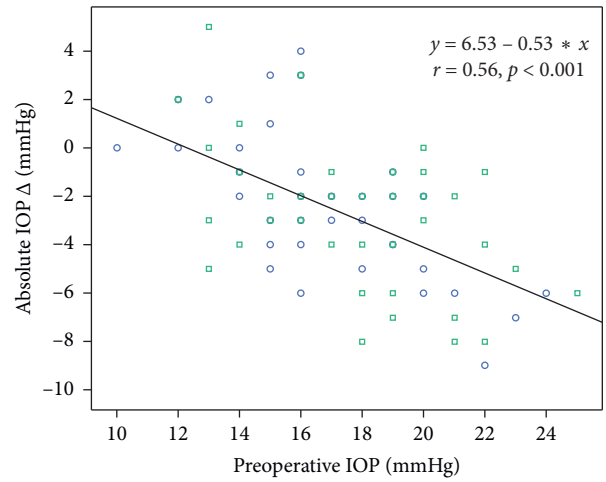

Figure 1: Scatterplot showing a linear relationship between preoperative IOP and absolute IOP change in both DM and non-DM groups. DM, diabetes mellitus; IOP, intraocular pressure. x = preoperative IOP; y = absolute IOP Δ; r = Pearson's correlation coefficient. Circles, non-DM subjects; squares, DM subjects.

3.2.3. Anterior Chamber Volume (ACV) Comparisons. Mean preoperative ACV was 126.4 ± 33.3 mm^3 and 138.0 ± 35.4 mm^3 in DM and non-DM groups, respectively ($p = 0.116$). ACV was observed to be significantly greater than preoperative value at 1 and 6 months of follow-up in both groups ($p < 0.001$, paired t-test). No group differences were observed with regard to ACV variations at 1 and 6 months after surgery (Table 2).

3.2.4. Anterior Chamber Angle (ACA) Comparisons. Mean preoperative ACA in DM group was significantly lower compared with non-DM group (30.2 ± 5.5° vs. 33.0 ± 5.9°, respectively ($p = 0.022$)). ACA was observed to

Table 3: Subgroup analysis of DM and non-DM groups according preoperative IOP subgroups.

	Pre-op. IOP	Eyes (n, %)	Age (y)	IOP pre-op. (mmHg)	IOP Δ 6 mo (mmHg)	ACD pre-op (mm)	ACD Δ 6 mo (mm)	ACV pre-op (mm^3)	ACV Δ 6 mo (mm^3)	ACA pre-op (°)	ACA Δ 6 mo (°)
DM group ($n = 45$)	21–25	9 (20%)	73 ± 7	22.0 ± 1.3	−4.8 ± 2.7	2.5 ± 0.4	+1.5 ± 0.2	129 ± 41	+59 ± 13	30 ± 5	+15 ± 7
	19-20	11 (24%)	72 ± 4	19.4 ± 0.5	−3.3 ± 2.3	2.5 ± 0.3	+1.2 ± 0.4	118 ± 22	+46 ± 21	29 ± 5	+13 ± 5
	17-18	9 (20%)	70 ± 7	17.7 ± 0.5	−4.1 ± 2.3	2.5 ± 0.3	+1.4 ± 0.2	119 ± 33	+55 ± 21	29 ± 4	+15 ± 5
	15-16	7 (16%)	72 ± 4	15.6 ± 0.5	−1.7 ± 2.1	2.5 ± 0.4	+1.5 ± 0.3	122 ± 35	+59 ± 27	27 ± 5	+16 ± 3
	10–14	9 (20%)	77 ± 4	13.3 ± 0.7	−0.4 ± 3.2	2.8 ± 0.3	+1.3 ± 0.2	146 ± 35	+46 ± 29	35 ± 7	+12 ± 5
	p	0.676[1]	0.140[2]	<0.001*[2]	0.028*[2]	0.320[2]	0.109[2]	0.401[2]	0.694[2]	0.088[2]	0.416[2]
Non-DM group ($n = 44$)	21–25	4 (9%)	76 ± 12	22.5 ± 1.3	−7.0 ± 1.4	2.6 ± 0.4	+1.3 ± 0.2	116 ± 23	+50 ± 27	29 ± 4	+9 ± 5
	19-20	8 (18%)	70 ± 5	19.5 ± 0.5	−3.4 ± 2.1	2.7 ± 0.5	+1.3 ± 0.3	145 ± 41	+39 ± 31	35 ± 6	+11 ± 8
	17-18	10 (23%)	70 ± 6	17.6 ± 0.5	−2.7 ± 1.0	2.7 ± 0.3	+1.3 ± 0.3	139 ± 24	+49 ± 16	35 ± 5	+12 ± 5
	15-16	15 (34%)	70 ± 6	15.5 ± 0.5	−1.8 ± 3.1	2.6 ± 0.4	+1.4 ± 0.3	135 ± 35	+53 ± 22	31 ± 6	+15 ± 5
	10–14	7 (16%)	71 ± 4	12.7 ± 1.5	+0.1 ± 1.5	2.8 ± 0.5	+1.3 ± 0.4	152 ± 48	+52 ± 29	34 ± 6	+14 ± 7
	p	0.759[1]	0.928[2]	<0.001*[2]	0.001*[2]	0.797[2]	0.763[2]	0.469[2]	0.715[2]	0.143[2]	0.516[2]

Data were derived from Fisher's exact test[1] and Kruskal–Wallis test[2]. Continuous variables are reported as mean ± standard deviation. *$p < 0.05$, statistical significance. ACA, anterior chamber angle; ACD, anterior chamber depth; ACV, anterior chamber volume; IOP, intraocular pressure; mm, millimeters; mo, months; Δ, variation.

Table 4: Uni- and multivariate regression analyses of the relative effects of the baseline variables on postoperative IOP change.

Parameter	Absolute IOP Δ (mmHg)			
	Univariate		Multivariate	
	B (95% CI)	p	B (95% CI)	p
Age (y)	−0.003 (−0.10 to +0.10)	0.960	−0.16 (−0.11 to +0.08)	0.747
Gender (female)	+0.84 (−0.39 to 2.07)	0.177	+0.89 (−0.34 to +2.12)	0.155
DM	−0.50 (−1.71 to +0.70)	0.410	−0.059 (−1.14 to +1.02)	0.914
Axial length (mm)	+0.06 (−0.75 to +0.87)	0.888	−0.12 (−1.07 to +0.82)	0.795
Pre-op CCT (μm)	−0.02 (−0.03 to +0.003)	0.102	−0.01 (−0.02 to +0.01)	0.589
Pre-op IOP (mmHg)	−0.53 (−0.70 to −0.36)	<0.001*	−0.53 (−0.88 to −0.19)	0.003*
PD ratio	−0.72 (−1.03 to −0.40)	<0.001*	−0.02 (−1.51 to +1.46)	0.976
PV ratio	−17.72 (−28.29 to −7.14)	0.001*	−8.02 (−42.09 to +26.04)	0.640
PA ratio	−6.41 (−9.87 to −2.95)	<0.001*	+2.64 (−4.69 to +9.96)	0.476

Data were derived from linear regression models. Continuous variables are reported as mean ± standard deviation. *$p < 0.05$, statistical significance. CCT, central corneal thickness; DM, diabetes mellitus; IOP, intraocular pressure; PA, pressure to angle ratio; PD, pressure to depth ratio; PV, pressure to volume ratio; mm, millimeters; y, years. The remaining variables (DM duration, HbA1c levels, CDE, cataract grade, and IOL type) did not influence the model and were excluded.

be significantly greater than preoperative value at 1 and 6 months of follow-up in both groups ($p < 0.001$, paired t-test), but no group differences were observed at final visit. Similarly, there were no statistical differences in ACA variations at 1 and 6 months after surgery (Table 2).

3.3. Factors Influencing the Postoperative IOP Change. Multivariate linear regression adjusting for age, gender, axial length, diabetes mellitus, CDE, and relevant AS Scheimpflug parameters (CCT, PD, PV, and PA) showed that only preoperative IOP was significantly associated with absolute IOP reduction 6 months after surgery. IOP was found to significantly decrease on average 0.53 mmHg for every 1 mmHg increase in preoperative IOP ($p = 0.003$; Table 4).

4. Discussion

Given the variability of the postoperative IOP response after uneventful phacoemulsification cataract surgery with posterior chamber IOL implantation reported in the literature [1], there has been a significant effort to understand the mechanisms underlying IOP changes. The information derived from basic and clinical studies has suggested that this is a multifactorial phenomenon that includes a reduction in aqueous production [23] and an improved conventional [21, 25, 26] and uveoscleral aqueous humor outflow [27].

Results from this study showed a comparable IOP reduction 6 months after cataract surgery in nonglaucomatous eyes with open angles from nondiabetic (−2.4 ± 2.8 mmHg) and type 2 diabetic patients (−2.9 ± 2.9 mmHg). In line with previous studies assessing AS morphometry changes by Scheimpflug imaging (Table 5), all eyes from both groups experienced a significant widening of the anterior chamber depth, volume, and angle, while mean CCT did not change significantly at 1 and 6 months after cataract surgery [8, 28, 29]. It should be noted that subjects' characteristics (age and ethnic differences), Scheimpflug devices (Pentacam CES [8, 28, 29] and HR [9], EAS-1000 [2], Sirius [30, 31]), and image analysis techniques were not the same in all studies. Therefore, precaution is warranted regarding direct comparisons between the studies.

Regarding postoperative ACD assessment [8, 20], the authors confirmed that the automatic analysis provided by the Pentacam software frequently resulted in erroneous measurements due to inaccuracies in the identification of IOL's anterior surface. In the current study, similarly to Dooley et al. [8], all postoperative measurements were performed manually by one of the authors. This method has been shown to have adequate repeatability and reproducibility in pseudophakic eyes [32]. Other Scheimpflug-based studies relied on the automatic evaluation [28] or did not specify the method used [9, 29].

Several aspects of anterior segment anatomy have been found to differ between DM and non-DM patients. Previous studies [12, 13] reported that diabetic subjects had shallower anterior chambers, probably secondary to an increased lens thickness. The present study confirmed that DM subjects have smaller anterior chamber angles; however, due to the relatively small population sample, the ACD and ACV differences did not reach statistical significance. Unfortunately, in our study, none of the technologies used was able to measure lens vault or thickness, and, so the influence of these important parameters on the ACD could not be ascertained.

In some population-based studies, diabetic patients had statistically significant higher IOP-GAT readings compared to nondiabetics. This finding has been attributed to an increased corneal thickness and stiffness caused by protein cross-linking resulting from advanced glycosylated end-products [14]. Moreover, Last et al. hypothesized that an elevated corneal resistance factor measured with the Ocular Response Analyzer®, as found in DM subjects, could be accompanied by an increased stiffness of the trabecular meshwork which, in turn, would cause greater resistance to aqueous humor outflow and IOP elevation [33]. In our study, the wide standard deviations of the IOP measurements or a relatively small sample size of the study populations could explain the lack of statistical differences in IOP readings between groups.

In the current study, the authors were not able to found any statistical differences regarding IOP or AS variations at 1 and 6 months between DM and non-DM subjects. The results of our univariate and multivariate linear regression analyses, which were adjusted for potential confounders,

Table 5: Review of studies assessing the IOP reduction and AS changes after phacoemulsification by Scheimpflug imaging.

Study (year)	Patients (eyes)	Glaucoma	Age (y)	Female (*n*)	Follow-up	IOP pre-op (mmHg)	IOP Δ (mmHg)	ACD pre-op (mm)	ACD Δ (mm)	ACV pre-op (mm^3)	ACV Δ (mm^3)	ACA pre-op (°)	ACA Δ (°)
Hayashi et al. [2] (2000)[a]	77 (77)	ACG	74 ± 8	56 (73%)	12 mo	21.4 ± 3.9	−6.1 ± 3.9*	1.9 ± 0.3	+2.1 ± 0.4*			19 ± 4	+18 ± 5*
	73 (73)	OAG	74 ± 7	39 (53%)		20.5 ± 5.4	−4.4 ± 4.3*	2.8 ± 0.4	+1.5 ± 0.4*	n.r.	n.r.	28 ± 5	+10 ± 7*
	74 (74)	No	72 ± 11	40 (54%)		17.3 ± 3.3	−1.0 ± 4.1*	2.9 ± 0.4	+1.4 ± 0.6*			27 ± 6	+11 ± 8*
Uçakhan et al. [28] (2009)[b]	44 (44)	No	66 ± 8	n.r.	3 mo	15.8 ± 3.7	−2.6 ± n.r.*	3.0 ± 0.8	+0.9 ± n.r.*	165 ± 50	+36 ± n.r.*	36 ± 10	+6 ± n.r.*
Doganay et al. [29] (2010)[b]	34 (42)	No	65 ± 8	8 (24%)	6 mo	14.6 ± 2.5	−2.8 ± n.r.*	2.8 ± 0.4	+1.9 ± n.r.*	145 ± 44	+46 ± n.r.*	33 ± 6	+10 ± n.r.*
Dooley et al. [8] (2010)[b]	101 (101)	No	69 ± 11	62 (62%)	6 weeks	14.8 ± 3.1	−2.5 ± 3.2*	2.7 ± 0.4	+1.1 ± 0.5 *	142 ± 49	+54 ± 27*	30 ± 6	+13 ± 7 *
Mota et al. [9] (2011)[c]	30 (31)	No	73 ± 8	18 (58%)	1 mo	20.5 ± 4.4[e]	−3.9 ± 5.6*	2.8 ± 0.5	+1.5 ± 0.7 *	n.r.	n.r.	32 ± 6	+13 ± 6 *
Takmaz et al. [30] (2012)[d]	54 (56)	No	66 ± 10	30 (56%)	1 mo	14.6 ± 3.5	−4.2 ± n.r.*	2.7 ± 0.4	+0.8 ± n.r.*	144 ± 49	+49 ± n.r.*	42 ± 8	+11 ± n.r.*
Şimşek et al. [31] (2016)[d]	132 (132)	No	64 ± 13	46 (35%)	3 mo	14.7 ± 2.6	−2.4 ± n.r.*	2.8 ± 0.4	+0.7 ± n.r.*	125 ± 26	+38 ± n.r.*	42 ± 7	+9 ± n.r.*

Statistically significant difference at $p < 0.05$. AS, anterior segment; CCT, central corneal thickness; n.r., not reported; SD, standard deviation; SE, standard error; US, ultrasound pachymetry; y, years; mmHg, millimeters of mercury. [a]AS evaluation with EAS-1000 (Scheimpflug videophotography system); [b]AS evaluation with Pentacam CES; [c]AS evaluation with Pentacam HR; [d]AS evaluation with Sirius; [e]IOP was measured with ocular response analyzer.

suggested no relationship between the presence of DM and long-term postoperative IOP reduction. Interestingly, at 1-month follow-up, diabetic patients had a smaller non-statistically significant reduction of IOP compared to the non-DM group, but this relationship was inversed at 6 months. Wang et al. [25] proposed that ultrasonic vibrations from phacoemulsification could induce stress remodeling of the trabecular meshwork and then lead to IOP reduction. It is possible that, in diabetic patients, this remodeling is delayed due to the overexpression of fibronectin induced by hyperglycemia [15].

Despite the growing recognition of the importance of preoperative IOP in postoperative IOP changes following phacoemulsification, only in 2008 Poley et al., by stratifying preoperative pressures, demonstrated that postoperative IOP reduction was proportional to preoperative IOP [21]. The present study, adopting the same methodology, allowed the authors to conclude that eyes with the highest mean preoperative IOP had the greatest magnitude of decrease and eyes with the lowest mean preoperative IOP had an insignificant mean IOP reduction or a mild IOP elevation [21–23]. Not only that, but it also showed that AS changes did not differ significantly between the subgroups, which suggest that preoperative IOP is the major factor that determines IOP reduction after phacoemulsification.

Predictive models of IOP reduction based on preoperative factors represent an important attempt to improve decision-making process of cataract surgery, in particular for ocular hypertension or glaucoma subjects with open-angles. Anterior segment-specific factors, including anterior chamber anatomy (depth [7, 8, 10, 11, 31], volume [8, 23, 31], and angle [4, 8, 23, 34]), iris (cross-sectional area and convex shape) [35], and lens factors (thickness [23], position [10, 11], and vault [5, 34]) are likely important predictors of the expected IOP change. However, the clinical significance and relationship between those variables continue to be controversial. In our study, PD, PV, and PA ratios were significantly associated with postoperative IOP change in univariate analysis; however, the effect was no longer significant after multivariate adjustment [5, 23]. In the multivariate model, the only significant predictor of postoperative IOP changes was preoperative IOP [36, 37].

Few studies have investigated the impact of phacoemulsification parameters on postoperative IOP changes. Similar to Lee et al. [17], our analysis failed to demonstrate any significant relationship between the amount of CDE and the IOP variations. A study by DeVience and colleagues [38] was able to show a significant correlation between phacoemulsification time and postoperative IOP reduction 24 months postoperatively. However, these findings were not confirmed by Pradhan et al. [35].

Limitations to this study include IOP measurement at a single visit preoperatively [37]. Also, the inclusion of cataract surgeries performed by multiple surgeons may have introduced some variability; nevertheless, no significant intersurgeon differences were observed. Another drawback is the fact that the present study excluded subjects with more advanced stages of DR (NPDR with maculopathy and PDR), mature cataracts, and complicated surgeries; therefore, we cannot make any considerations in those particular groups of patients. Finally, only Caucasian patients were included.

In conclusion, this study found that IOP reduction 6 months following uneventful phacoemulsification was strongly correlated with preoperative IOP in non-glaucomatous eyes with open-angles, without any difference between DM and non-DM groups. Additional studies may support our findings, and this topic needs further evaluation, inclusive with other AS imaging devices.

Data Availability

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

Disclosure

This study was presented in XXXV Congress of the European Society of Cataract and Refractive Surgeons, 7–11 October 2017, Lisbon, Portugal, and 60th Portuguese Ophthalmology Congress, 7–9 December 2017, Vilamoura, Portugal.

Conflicts of Interest

Manuel Falcão has participated in advisory boards for Bayer and has received travel grants from Novartis, Alimera, and Allergan. Angela Carneiro has participated in advisory boards for Alcon, Bayer, Novartis, Alimera, Allergan, and Roche. The other authors declare no conflicts of interest regarding the publication of this paper.

Authors' Contributions

JNB, MF, VR, AC, and FFR were involved in the study concept and design. JEL and DR were responsible for data collection. JNB, JEL, MF, AC, VR, and FFR were responsible for analysis and interpretation of data. JNB, JEL, and DR drafted the manuscript. MF, VR, AC, and FFR critically revised the manuscript. JNB and MF provided statistical expertise.

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Review Article

An Update on Corneal Biomechanics and Architecture in Diabetes

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In the last decade, we have witnessed substantial progress in our understanding of corneal biomechanics and architecture. It is well known that diabetes is a systemic metabolic disease that causes chronic progressive damage in the main organs of the human body, including the eyeball. Although the main and most widely recognized ocular effect of diabetes is on the retina, the structure of the cornea (the outermost and transparent tissue of the eye) can also be affected by the poor glycemic control characterizing diabetes. The different corneal structures (epithelium, stroma, and endothelium) are affected by specific complications of diabetes. The development of new noninvasive diagnostic technologies has provided a better understanding of corneal tissue modifications. The objective of this review is to describe the advances in the knowledge of the corneal alterations that diabetes can induce.

1. Introduction

The first World Health Organization (WHO) global report on diabetes mellitus indicates that the number of adults living with this disorder has almost quadrupled since 1980 to 422 million adults. This large increase is due mainly to a higher incidence of type 2 diabetes (T2D) and the influence of factors such as overweight and obesity [1]. Diabetes is a systemic metabolic disease associated with high morbidity and mortality that can affect almost all tissues of the human body, including the most superficial and transparent ocular tissue: the cornea [2–7]. The prolonged high blood glucose levels that occur in diabetes can cause severe ophthalmological complications that affect both the anterior and posterior segments of the eye and can produce a significant visual deficit, including blindness. The eyeball is an organ accessible to noninvasive exploration and can provide great information about the possible involvement of other systemic organs caused by diabetes. The different corneal components (epithelium, stroma, nerves, and endothelium) are each affected by specific complications related to diabetes and poor glycemic control. It is well known that diabetic retinopathy is a good indicator of the state of microvascular disease in the rest of the organs. In the same way, the changes in corneal structures that we can recognize with new noninvasive technologies could predict systemic complications of diabetes or evaluate control of the disease. These changes in the corneal nerves of patients with diabetes could predict systemic conditions such as peripheral and autonomic neuropathy, while the state of the endothelial cells or

changes in corneal thickness could inform on the status and level of control of the disease. The possibility of identification of structural and biomechanical changes of the cornea in patients with diabetes by means of accessible and non-invasive techniques can offer a new possibility for the early treatment of possible systemic complications. An improved knowledge of the changes produced by diabetes in the cornea and advances in diagnostic technology made in the last 10 years have led to substantial progress in our understanding of the biomechanics and architecture of the cornea. This review summarizes advances in our knowledge of the clinical manifestations and the "layer by layer" corneal changes that diabetes can produce.

2. Materials and Methods

We have carried out a systematic review of the literature published between January 1, 2008 and November 1, 2018 concerning the role of diabetes in structural and biomechanical changes in the cornea. A literature search was conducted in the NCBI Entrez PubMed database combining the term "diabetes" with a series of key words such as "corneal epithelium," "corneal thickness," "corneal stroma," "corneal biomechanics," "ocular response analyzer," "corneal hysteresis," "corneal nerves," and "corneal endothelium." Of the 314 manuscripts registered initially, those that were duplicated or without a summary in English were excluded, and 243 articles were finally examined by the coauthors to determine their relevance. The articles that included only the posterior segment were considered not relevant. A total of 81 papers were deemed irrelevant.

3. Diabetes and the Corneal Epithelium

Diabetes is associated with ocular surface disorders such as dry eye, superficial punctate keratitis, recurrent corneal erosion syndrome, and persistent epithelial defects [8, 9]. The underlying and responsible mechanisms that have been suggested for the appearance of these pathologies are a loss of corneal innervation (see Corneal Nerves in Diabetes), loss of basal epithelial cells, production and accumulation of advanced glycation end products (AGEs), disruption of tight junctions between epithelial cells, and disruption of trophic factors that encourage wound healing.

3.1. Basal Epithelial Cell Density (BECD). Cai et al. [10] evaluated the effects of type 1 diabetes (T1D) on the whole cornea, corneal sublayer thickness, and basal epithelial cell density (BECD) using in vivo corneal confocal microscopy (CCM) in a streptozotocin-induced diabetic mouse model. They found reduced BECD and a decreased thickness of the corneal epithelium in these diabetic mice. Dehghani et al. [11] reported a decrease in the thickness of basal and intermediate epithelial cell density in a human in vivo case-control study with laser-scanning CCM in a cohort of diabetic patients. Similar results were obtained by Szalai et al. [12] and Qu et al. [13], who also found a significant decrease in the cell population of the basal epithelial layer. Different mechanisms have been proposed as causal for this outcome, including decreased innervation at the subbasal nerve plexus (SBNP) (see Corneal Nerves in Diabetes), increased basement membrane thickness, or metabolic dysfunctions associated with the accumulation of AGEs in the basal membrane [11, 14].

3.2. Epithelial Basement Membrane. Classically, diabetes has been associated with corneal epithelial basement membrane (BM) disorders [15–17]. BM becomes irregularly thickened and multilaminated, with abnormal adhesions to the supralying epithelium [18], and has been related to accumulation of AGEs. This enlarged configuration of the basal membrane leads to subclinical scattering of light in the cornea visible on in vivo CCM, but not detectable on routine clinical examination [19, 20]. Recently, Özyol and Özyol [21], by using Scheimpflug tomography in a cohort of diabetic patients scanned by densitometry, detected that the anterior corneal layer displayed significantly higher values on light scattering in diabetic eyes than in the eyes of controls.

Regarding the biochemical changes in the composition of the corneal BM, Ljubimov et al. [17, 22] reported a markedly diminished change with a weak staining for chains of laminin-l, entactin/nidogen, laminin-10, and a3-a4 chains of type IV collagen in diabetic corneas with diabetic retinopathy. Saghizadeh et al. [23] also found reduced immunostaining of laminins, entactin/nidogen-1, and laminin receptor integrin $\alpha 3\beta 1$. In addition, they report a significant decrease in the laminin $\gamma 3$ chain and fibronectin [24]. Different hypotheses could be responsible for these changes in the composition of the corneal BM, an increase in the activity of the proteinases, and a decrease of growth factors or diffusion from the vitreous or the retina of pathological substances associated with hyperglycemia may vary the composition of the corneal BM. Moreover, it has been suggested that changes in the composition of the corneal BM in diabetic patients could alter the interaction between epithelial cells and the underlying basal membrane, triggering variations in the expression patterns of integrins [25].

3.3. Tight Junctions. The major function of the corneal epithelium is to protect the interior of the eye; the corneal epithelium creates "tight junctions"—physical and chemical barriers that protect against infection, maintaining corneal transparency and integrity. Epithelial cell junctions, visualized as electron dense structures, play an important role in the formation and maintenance of the epithelial barrier, homeostasis, and host defense of the cornea.

Huang et al. [26], using a diabetes rat model, found delayed corneal healing with fewer multilayers of epithelium covering the denuded surface at 48–72 hours, with increased disorganization of occludin protein stained with immunofluorescence. Scanning electron microscopy revealed abnormal intercellular connections, fissures between cells, a decrease in the number of microvilli, and dropsy in the diabetic rat group. Yin et al. [27] reinforced this idea when they observed a delayed, but not absent, formation of tight junctions between cells during the healing process of

epithelial corneal ulcers in diabetic rats. There are no studies in humans that corroborate these findings in animal models.

3.4. Advanced Glycation End Products (AGEs). AGEs have been proposed as the cause of the abnormalities seen in the cornea of patients with diabetes. They are a heterogeneous group of substances that result from the nonenzymatic glycation and oxidation of proteins and lipids. AGEs stimulate cell apoptosis by increasing intracellular reactive oxygen species (ROS) production [28, 29].

The accumulation of AGEs leads to alterations in tissue function. AGE accumulation has been detected at the site of the corneal epithelium and epithelial BM in diabetic rats [30, 31] and monkeys [32] and in human diabetes patients [29]. In addition, it has been shown that the AGE concentration is elevated in the tears of human diabetes patients [33]. Kim et al. [30] demonstrated both the accumulation of AGEs and the presence of oxidative DNA damage in diabetic corneal cells. They found a correlation between the apoptotic damage in the diabetic cornea and the intense nuclear localization of a marker of oxidative DNA damage (8-hydroxydeoxyguanosine). These findings provide strong evidence that nuclear oxidative DNA damage by AGE accumulation is responsible, at least in part, for the apoptotic damage of diabetic corneal cells, leading to delayed epithelial wound healing in the diabetic cornea.

3.5. Wound Healing. Several authors have recently demonstrated delayed wound healing in diabetic rat models [27, 34, 35]. Longer healing times than those in the control group were observed in a group of diabetic rats in which a mechanical debridement had been performed. Growth factors and cytokines are powerful regulators of cell behavior and promote tissue wound healing. Disruption of trophic factors has been identified as being responsible for delayed corneal healing in both human and animal models of diabetes. An important example is epidermal growth factor receptor (EGFR); this pathway is critical for cell migration and proliferation and is a major mediator of corneal epithelial wound healing [36]. Several authors have reported disruption of this pathway in the cornea of diabetic rats [27] and in human corneal epithelial cells [37, 38].

Another altered pathway is mediated by hepatocyte growth factor (HGF) which is involved in the processes of cellular proliferation, migration, and apoptosis [24, 39] The HCG receptor, the proto-oncogene c-Met, is apparently involved in activation of p38 mitogen-activated protein kinase (p38 MAPK) which has been related to stimulation of corneal epithelial migration [40]. Saghizadeh et al. reported an increased expression of HGF and a diminished c-Met expression in the diabetic cornea [41]. Recent studies carried out by the same group of researchers have developed an adenoviral-based gene therapy in human diabetic cultured corneas, improving wound healing times by normalizing the levels of c-Met expression, associated or not with the normalization of other proteinases or kinases whose values are usually altered in the corneas of diabetic patients [24, 41–44].

Other routes which have recently been studied include Serpine 1 [35], which, when compared to controls, is significantly diminished in corneal epithelium collected from diabetic rats. In addition, opioid growth factor (OGF) [45, 46], which is elevated in the plasma of patients with diabetes, acts as a negative regulator of epithelial proliferation and wound healing. When OGF joins to its specific receptor, OGFr, they are able to inhibit cell replication [46]. Moreover, it has been observed that opioids antagonists such as naltrexone, which block the axis OGF-OGFr, favor cell replication and therefore tissue remodeling [45].

Likewise, insulin-like growth factor sun-1 (IGF-1) and its receptor, which are found in human corneal keratocytes and epithelial cells, mediate cell migration, proliferation, and survival. It appears that elevated levels of insulin-like growth factor binding protein 3 (IGFBP-3) found in the tears of diabetic human subjects may attenuate IGF-1 receptor signaling in the diabetic cornea [47]. According to Wang et al. [48,49], this attenuation via IGFBP3/IGF-1 could be promoted by Sirtuin 1 (silent mating type information regulation 2 homolog), a protein that belongs to the group of class III histone/protein deacetylases. In addition, Shen et al. [50] reported that corneal wounds in diabetes have abnormal electric signals which may contribute to impaired wound healing, possibly via cell electrotactic migration disruption, and even suggest electrical stimulation as a new therapeutic option in the management of chronic and nonhealing wounds.

4. Diabetes and Corneal Stroma

4.1. Corneal Nerves in Diabetes. The structure of the corneal nerves is very important in maintaining a healthy ocular surface. The cornea is the most densely innervated tissue in the human body (approximately 7,000 nociceptors per mm^2) [51]. This great sensitivity serves to protect the cornea. The corneal nerves are derived from the ciliary nerves that form the terminal branches of the ophthalmic division of the 5th cranial nerve. These bundles of nerves penetrate radially in the middle and anterior corneal stroma through the limbus and then bifurcate and advance towards the epithelium as long bundles, fine branches, and nerve terminals [52]. This results in a moderately dense midstromal plexus and a dense subepithelial plexus, whose branches cross Bowman's membrane to form an SBNP complex that emits nerve terminals that innervate all epithelial layers [53]. The different types of nerve endings (nociceptive, temperature, and polymodal) are responsible for sensations such as pain, touch, temperature, and dryness, which are very important for the reflex of blinking, the production of tears, and the healing of lesions [54–58].

Diabetes is a systemic condition that can affect corneal innervation and sensitivity, causing complications that can lead to blindness. Patients with diabetes show a reduction in corneal sensitivity, clinically measured with an esthesiometer [59], due to a progressive decrease in the density of the corneal nerves [60]. Advances in technology have allowed for rapid, noninvasive, and high-quality visualization of the corneal structure using in vivo CCM. The corneas of patients with diabetes show a lower density of SBNP, a

reduction of epithelial nerve fiber bundles per image with decreased branches, and greater nervous tortuosity than the corneas of healthy patients [61, 62]. These alterations are associated with a reduction in corneal sensitivity in patients with diabetes [63]. He and Bazan studied the architecture of corneas donated by patients with insulin-dependent diabetes of varying duration. Although they did not find differences in the number of nerve trunks of the stroma, they found a decrease in the density of epithelial nerves in the corneas of patients with 5 or more years' duration of insulin-dependent diabetes. The presence of abundant loops of nerve fibers in the corneal stroma, which appeared to be formed as a result of resistance in the BM to the penetration of the stromal nerve branches in the epithelia, was also observed [64].

Damage to the corneal nerve fibers leads to an alteration of the healing process of the wounds and greater susceptibility to infections; this damage causes most of the symptoms experienced by diabetes patients with keratopathy, such as decreased corneal sensitivity, recurrent corneal erosions, persistent epithelial defects, and neurotrophic corneal ulcers [65–67].

Examination of the corneal nerves and exploration of corneal sensitivity are useful tools for the early detection and evaluation of peripheral neuropathy in patients with diabetes. Several studies have shown that CCM is a valid, accurate, noninvasive method to identify small nerve fiber pathology; CCM can also be used to diagnose diabetic neuropathy [68, 69]. It has been found that corneal nerve fiber density and length, as well as corneal nerve branch density, are significantly reduced in patients with diabetic polyneuropathy when compared to control subjects. The diagnostic efficiency of CCM is comparable to intraepidermal nerve fiber density by skin biopsy; however, CCM may be preferred due to its rapid, automated, and noninvasive characteristics [69]. What is important to recognize is that CCM can identify nerve alterations in the cornea that precede the clinical signs and symptoms of peripheral neuropathy, nephropathy, or diabetic retinopathy. Asghar et al. observed alterations even in patients with impaired glucose tolerance but who did not meet the clinical criteria of T2D [70]. CCM is also useful in the assessment of a patient's response to treatments, since it has been found that there is a recovery of corneal SBNP and an improvement of neuropathy in diabetes patients who have received a double pancreas and kidney transplant [71, 72]. A recent study using in vivo CCM has found that nerve fiber damage in T1D correlates with the degree of diabetic retinopathy. Furthermore, studies show that T1D patients with higher age at diagnosis have a higher nerve fiber density. These results indicate that age at T1D diagnosis potentially has an important effect on final nerve fiber density [73]. In conclusion, studies show that CCM offers an early, faster, and less invasive diagnosis of diabetic peripheral neuropathy than current gold standard techniques such as nerve electrophysiology, sural nerve biopsy, and skin puncture biopsy.

4.2. Corneal Stroma Structure and Biomechanics in Diabetes. The stroma represents 90% of the corneal thickness; its special structure and composition give the cornea its biomechanical properties [74]. The highly differentiated ultrastructure of the corneal stroma, with its special orientation, diameter, and separation of fibrillar collagen bundles and the regulatory role of other components of the extracellular matrix (proteoglycans and glycosaminoglycans), confer transparency and biomechanical behavior to the cornea [75, 76]. The way in which diabetes affects the structure and function of the corneal stroma is not well known; there have been numerous studies in recent years into how diabetes affects corneal thickness and the biomechanical properties of the corneal stroma. The main points of interest in the reviewed papers on corneal biomechanics in diabetes involved the in vivo measurement of the corneal biomechanical properties; this was largely due to the recent development of technological devices to quantify some of these properties. The first of these was the Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY, USA), and more recently the Corvis ST (Corvis ST; Oculus, Wetzlar, Germany). In addition, in the last two years, details of corneal optical densitometry (COD) analysis using the Pentacam HR imaging system in diabetes patients have been published.

4.2.1. Corneal Thickness. Recently published research findings on corneal morphology show evidence of greater central corneal thickness (CCT) in patients with T2D [77–80]. In studies of corneal thickness in patients with diabetic retinopathy, no statistical differences were found between groups of patients with proliferative retinopathy or nonproliferative retinopathy and those without diabetic retinopathy [81–85]. These results indicate that diabetes patients have a significantly thicker CCT, regardless of the state of retinopathy. Santiagu et al. [86] found that diabetes during pregnancy also does not seem to influence CCT. In a recent article, Kumar et al. [87] showed that CCT increases in relation to the severity of peripheral diabetic neuropathy due to an increase in stromal thickness. Other studies, however, have not found an increase in CCT in cases of T1D [88] or T2D patients [89–91]. Similarly, studies of patients with primary open-angle glaucoma (POAG) did not show differences in CCT between groups of glaucoma patients with and without diabetes [92, 93]. Hashemi et al. [94], in a five-year follow-up study, showed that overall patterns of change in CCT and corneal shape in diabetes patients over 40 years of age were similar to those observed in those individuals without diabetes. However, changes related to age in the thickness, volume, and shape of the central and peripheral cornea were less pronounced in subjects with diabetes.

Several studies on corneal thickness and biomechanics have been conducted in children with T1D. Tiutiuca [95] conducted a study in 100 children with T1D in Romania that showed an increase in CCT when compared to an equivalent number of healthy children. These results are comparable to those from a similar study conducted in Turkey by Akinci et al. [96]. However, other studies have not found this increase in CCT in children or young people with T1D [97, 98]. In another Turkish study in children with T1D, CCT was not shown to be associated with either the current fasting

glucose level or duration of disease [99]. However, in a recent clinical paper on corneal thickness in T1D, higher CCT values were observed in acute hyperglycemia state, when compared with those obtained after 48 hours of metabolic compensation, concluding that corneal pachymetry can potentially serve as a promising method for noninvasive evaluation of the increased risk of developing cerebral edema in patients with T1D [100].

4.2.2. Biomechanical Properties. ORA and Corvis ST are noncontact devices that provide tonometry and corneal displacement measurements via the injection of a rapid jet of air. ORA was the first device capable of evaluating in vivo biomechanical properties such as corneal hysteresis (CH) and corneal resistance factor (CRF), calculated from the differences in pressures that act to achieve defined corneal deformation states. In addition, ORA provides the intraocular pressure (IOP) correlated with the Goldmann IOP (IOPg) and the compensated corneal IOP (IOPcc). CH predominantly reflects the viscous properties of corneal tissue, whereas CRF is an empirically derived measurement representative of the elastic properties of the cornea [101]. Both parameters are derived from a complex interaction between the collagen composition of the cornea, its thickness, hydration, age, and other physiological factors [102, 103]. Studies have shown that lower CH values may be associated with several disorders such as keratoconus, Fuchs endothelial corneal dystrophy, and glaucoma [104–106]. The measures provided by the ORA have not been affected by CCT values [107].

Table 1 summarizes the publications in the last ten years that concern biomechanical corneal properties measured with ORA in diabetes patients. In most of the cross-sectional studies reviewed, it has been found that subjects with diabetes have higher CH values than the population without diabetes [74, 91, 92, 108, 110, 112, 115–118]. Only three studies [109, 113, 114] reported that subjects with diabetes have a lower CH when compared to age-matched controls, and four others did not find significant differences in CH values between populations with and without diabetes [99, 111, 115, 119]. A possible relationship between increased CH and the control of diabetes has also been investigated. Kotecha et al. [110] found that the level of glucose in the blood correlated significantly (but weakly; $r = 0.28$) with Hashemi et al. [94] found that subjects with fasting blood glucose values greater than or equal to 7.0 mM had higher CH and CRF values than those with glucose values less than 6.1 mM. Regarding corneal biomechanical properties in diabetic children, two studies show that T1D does not have any effect on corneal biomechanical parameters (CH and CRF) in childhood [99, 111] (Table 1). We found only one study that analyzed the results of these biomechanical parameters measured with Corvis ST in a diabetes population: Perez-Rico et al. [113] found differences in some parameters of corneal deformation in the diabetic population, with an increase in the time of the first applanation and a significant decrease in some parameters, such as the time of second applanation, the velocity of the first applanation, and the maximum deformation amplitude at the corneal apex.

4.2.3. Intraocular Pressure (IOP). POAG patients, both with and without diabetes have also been studied using ORA. In a study by Castro et al. [92], in which 74 eyes of 44 POAG patients were evaluated, it was found that CH was significantly higher in POAG patients with diabetes compared to POAG individuals without diabetes, without finding differences in the CCT. CRF, diabetes duration, and the effect of metabolic control on corneal biomechanical properties were not evaluated in this study. More recently, Akkaya et al. [93], in a study of 101 eyes of 101 patients, found that CH in diabetes was similar, but CRF, mean rim area, and rim volume (measured by optical coherence tomography) were found to be significantly higher in POAG patients with diabetes when compared to POAG patients without diabetes (Table 1). The results of these studies could suggest a protective role of diabetes in patients with glaucoma.

Several studies indicate a relationship between diabetes and higher IOP values [78, 109, 112–114], but this association is controversial. On one hand, diabetes is associated with a thicker CCT, but a thick cornea also provides higher IOP values. Luo et al. [120], in an extensive study, assessed both the direct and indirect effect of diabetes on IOP through the CCT mediator. They found that diabetes was associated with higher IOP, and CCT only contributed in a small proportion to the total effect of diabetes on IOP. This direct association between diabetes and IOP may have a pathophysiological importance with respect to the risk of glaucoma in people with diabetes.

4.2.4. Corneal Densitometry. Some studies on COD analysis using the Pentacam HR imaging system in diabetes have been recently published. COD is used to describe the characteristics of the corneal tissue and makes it possible to quantify its degree of transparency. Previous findings showed that COD in an area of inflammation was higher than normal, even when the damage was repaired [121]. It has also been confirmed that Pentacam HR objectively assesses a nubecula through a quantitative measurement of corneal density [122]. Gao et al. [123] used Pentacam HRto assess CCT, COD, and alterations of corneal transparency in 180 diabetes patients; they found an increase in COD and CCT compared with controls, with a positive association between the medial and intimal COD and central CCT in diabetes patients. In addition, Calvo-Maroto et al. [124], in a pilot study in adult diabetes patients, showed higher values of corneal light backscatter in patients with diabetes when compared with healthy subjects. However, COD values in children with T1D were similar in all concentric zones and layers to those in healthy children [125]. These findings suggest that there is an influence of the age and/or time of evolution of diabetes in the degree of corneal transparency or COD as determined by Pentacam HR.

4.2.5. Analysis of Findings. The reason why diabetes is associated with increased CCT in cases without corneal epitheliopathy is still unknown. It has been speculated that there may be an accumulation of AGEs in the corneal stroma of patients with diabetes, along with a nonenzymatic cross-linking between the collagen fibers and the proteoglycans.

Table 1: Summary of prospective cross-sectional studies of CH, CRF, IOPg, and IOPcc in diabetes patients.

Author, year, country	Study groups/sample size	Mean age (years)	ORA parameters (mean mmHg) controls/diabetes	Outcomes (P value)	Associations
Goldich, 2008, Israel [108]	40 with diabetes (40 eyes)/ 40 controls (40 eyes)	60.9/63.8	CH: 10.7 ± 1.6/9.3 ± 1.4 CRF: 10.9 ± 1.7/9.6 ± 1.6 IOPcc: 16.6 ± 4.4/17.7 ± 4.9 IOPg: 16.6 ± 4.3/16.1 ± 4.9	**0.0001** **<0.0001** 0.31 0.66	(i) Subjects with diabetes had **higher CH and CRF** values than those without diabetes (ii) There was **no any** statistical **difference** between the groups in terms of **IOPg and IOPcc**.
Sahin, 2009, Turkey [109]	43 with diabetes (81 eyes)/61 control (120 eyes)	55.3/53.1	CH: 9.51 ± 1.82/10.41 ± 1.66 CRF: 10.32 ± 1.76/ 10.36 ± 1.97 IOPcc: 18.81 ± 4.71/ 15.85 ± 3.24 IOPg: 17.68 ± 4.42/ 15.34 ± 3.66	**0.0001** 0.8 **0.0001** **0.0001**	**(i) CH** was found to be **significantly lower in diabetic** patients (ii) There was no significant difference in terms of **CRF** **(iii) Mean CCT, GAT, IOPg, and IOPcc were significantly higher in diabetic** patients than in healthy control subjects
Castro, 2010, Brazil [92]	44 **primary open-angle glaucoma patients**) 19 with diabetes (34 eyes)/ 25 without diabetes (40 eyes		**CH:** 9.1 ± 1.9/7.8 ± 1.7	**0.04**	Diabetic patients presented significantly **higher CH** values than patients without diabetes. There was a significant and positive correlation between CH and CCT for all patients ($r = 0.407$, $P < 0.001$).
Kotecha, 2010, UK [110]	61 with diabetes (61 eyes) T1D (13 eyes)/T2D (48 eyes)/controls (123 eyes)	41.9/ 61.6/54.0	CH: 12.45 ± 1.74/ 10.90 ± 1.94/10.85 ± 1.68 CRF: 12.49 ± 2.01/ 11.50 ± 2.06/10.62 ± 1.64	0.008 **0.0001**	(i) The **CH was significantly greater** in T1D patients. (ii) The **CRF was significantly greater** in T1D and T2D patients. (iii) CH and CRF were weakly correlated with blood glucose concentration
Kara, 2012, Turkey [99]	46 T1D children (46 eyes)/ 50 controls (50 eyes)	14.2/14.5	CH: 12.3 ± 1.3/12.5 ± 1.5 CRF: 12.4 ± 1.7/11.9 ± 1.5 IOPg: 17.4 ± 3.6/16.7 ± 2.9 IOPcc: 15.5 ± 3.4/15.1 ± 2.7	0.609 0.152 0.232 0.446	**(i) CH and CRF in T1D are similar** to those of healthy controls. (ii) IOPg and IOPcc in T1D are similar to those of healthy controls.
Nalcacioglu-Yuksekkaya, 2014, Turkey [111]	68 T1D children (68 eyes)/ 74 controls (74 eyes)	12.7/12.9	CH: 10.8 ± 1.5/10.7 ± 1.7 CRF: 10.9 ± 1.9/10.5 ± 1.6 IOPcc: 15.8 ± 3.0/15.3 ± 3.4 IOPg: 15.9 ± 3.7/15.2 ± 3.4	0.624 0.207 0.395 0.263	**(i) CH and CRF in T1D are similar** to those of healthy controls. (ii) IOPg and IOPcc in T1D are similar to those of healthy controls.

Table 1: Continued.

Author, year, country	Study groups/sample size	Mean age (years)	ORA parameters (mean mmHg) controls/diabetes	Outcomes (P value)	Associations
Yazgan, 2014, Turkey [112]	156 with T2D (156 eyes)/74 controls (74 eyes)	57.75/ 57.91	CH: 10.37 ± 1.9/8.98 ± 1.4	**0.0001**	**CH, CRF, CCT, IOPg and IOPcc values were higher in diabetes** groups than controls. There was also a positive correlation between HbA1C level and intraocular pressure.
			CRF: 11.06 ± 2.3/8.99 ± 1.5	**0.0001**	
			IOPg: 17.63 ± 3.9/14.80 ± 2.9	**0.0001**	
			IOPcc: 17.70 ± 3.2/ 16.56 ± 2.4	**0.026**	
Pérez-Rico, 2015, Spain [113]	94 diabetic patients (94 eyes)	59.8/62.2	CH: 10.23 ± 1.83/ 10.9 ± 1.39/11.43 ± 1.69	**0.002**	(i) **CH** was significantly **lower** in diabetic patients with elevated HbA1c than in controls and was affected by disease duration, whereas the CRF remained unaltered. (ii) **IOPcc and IOPg were significantly higher** in diabetic patients with elevated HbA1c than in controls.
	54 uncontrolled diabetes/40 controlled diabetes/41 controls		CRF: 11.05 ± 1.97/ 11.21 ± 1.97/10.53 ± 1.78	0.263	
			IOPcc: 18.45 ± 3.79/ 14.68 ± 2.67/14.55 ± 3.72	**<0.0001**	
			IOPg: 18.16 ± 3.85/ 15.31 ± 3.14/14.46 ± 4.1	**<0.0001**	
Schweitzer, 2016, France [91]	Diabetes (137 eyes)/controls (695 eyes)	—	**CH:** 9.79/9.28	**0.003**	Subjects with diabetes had **higher CH and CRF values** than those without diabetes. Consistently, subjects having fasting blood glucose values greater than or equal than 7.0 mM had significantly higher CH and CRF mean values compared with subjects having fasting blood glucose values lower than 6.1 mM ($P < 0.05$).
			CRF:10.35/9.63	**0.003**	
Akkaya, 2016, Turkey [93]	**101 primary open-angle glaucoma patients (101 eyes)**		**CH**: 9.35 ± 1.49/8.86 ± 1.52	0.11	(i) **CH in diabetes was similar** to those of healthy controls. (ii) RNFL thickness was measured by using Spectralis HRA + OCT. (iii) **CRF,** mean rim area, and rim volume were found to be significantly **higher** in the diabetic group when compared with nondiabetic group.
	60 with diabetes (60 eyes)/41 without diabetes (41 eyes)		**CRF:** 10.15 ± 1.78/ 9.24 ± 1.92	**0.01**	

Table 1: Continued.

Author, year, country	Study groups/sample size	Mean age (years)	ORA parameters (mean mmHg) controls/diabetes	Outcomes (P value)	Associations
Bekmez, 2018, Turkey [114]	50 with T2D (50 eyes)/50 controls (50 eyes)	63.3/61.7	CH: 9.9 ± 1.5/10.5 ± 1.7	0.080	(i) There was **no any** statistical **difference** between the groups in terms of **CH and CRF**. However, mean CH and CRF values were found less in diabetic group.
			CRF: 10.4 ± 1.6/10.5 ± 1.7	0.730	
			IOPcc: 17.8 ± 3.6/16.0 ± 3.1	**0.006**	(ii) Corneal biomechanical differences seen in diabetic patients may be associated with significantly higher IOP measurements.
			IOPg: 16.9 ± 3.5/15.4 ± 2.9	**0.032**	

T1D = type 1 diabetes; T2D = type 2 diabetes; ORA = ocular response analyzer; CH = corneal hysteresis; CRF = corneal resistance factor; GAT = Goldmann applanation tonometry; IOP = intraocular pressure; CCT = central corneal thickness; IOPg = Goldmann-correlated intraocular pressure; IOPcc = corneal-compensated intraocular pressure.

This cross-linking could theoretically explain the greater rigidity and thickening of the cornea in diabetics (higher CH, CRF, and CCT in some studies). Zou et al. [32] compared eight monkeys with insulin-dependent diabetes (induced by streptozotocin injection) with four controls, and found a cross-linking with abnormal aggregates of collagen fibrils in the stromal matrix on transmission electron microscope examination in monkeys with diabetes. In another recent experimental study in rabbits, Bao et al. [126] investigated the effects of diabetes on the behavior of the cornea, showing a significant increase in AGEs, CCT, and IOP in rabbits with diabetes. In addition, the tangent modulus of the cornea at four stress levels was significantly higher in rabbits with diabetes, indicated by greater mechanical rigidity of the cornea. These findings are consistent with evidence presented by Goldin et al. [127] in relation to the AGE-induced cross-linking of the extracellular matrix of certain tissues in patients with diabetes, which results in an increase in arterial stiffness. The fact that children with diabetes have the highest CCT without evidence of other systemic complications of diabetes suggests that AGEs may affect the cornea before other organs [95, 104] and that a test as accessible as pachymetry may be used to detect early changes.

The determination of corneal biomechanical properties can provide information on changes in the extracellular matrix in the eyes of diabetes patients and could therefore offer a new parameter for monitoring the state of the disease. In this review, we have found several studies conducted with ORA that have investigated the influence of diabetes on the biomechanical parameters of the cornea, but with somewhat contradictory results. Most of them (Table 1) find higher CH values in diabetes patients that could be caused by changes in the fundamental substance of the cornea, which would modify its viscosity [74, 108, 113, 115]. The oxidative stress caused by sustained hyperglycemia leads to the formation of AGEs (by nonenzymatic glycosylation) that accumulate in the tissues; in addition, a glycation of proteoglycans and glycosaminoglycans of the matrix is proposed, which would modify the viscosity of the cornea, increasing the CH [74, 115].

In addition, there are further pathogenic factors that could modify the biomechanical properties of the cornea in diabetes patients; these should be considered to clarify some contradictory results in the published evidence. A dysfunction of the epithelial and endothelial cells of the cornea could alter control of hydration of the cornea, causing subclinical edema that could influence the results by causing a decrease in CH and CRF, as well as an increase in CCT [74, 105, 126]. This hypothesis could explain the decreased CH values reported in some studies [109, 113, 114] and the elevated CCT in most of the studies [77–80]. Factors such as axial length [128], possible endothelial dystrophy [105], the existence of a subclinical keratoconus [129], or lubrication of the surface [130] can produce significant biomechanical changes that should be considered in future studies. In addition, to determine how the parameters would change during progression of the disease, measurement of the biomechanical properties in the same patients over time would be necessary. In future, we expect interesting findings regarding the biomechanical properties of the cornea in diabetes.

5. Diabetes and Endothelium

Table 2 summarizes the publications in the last ten years that concern endothelial status in diabetes patients, compared in most cases with healthy controls.

The italicized publications in Table 2 did not find statistically significant disagreement between the endothelial cell density (ECD) of diabetes patients when compared with healthy controls [72, 134, 135]. However, the majority of authors found differences in the endothelial cell population in individuals with versus without diabetes; the number of cells is decreased in diabetes patients, especially in those with T1D [12, 132, 133, 136]. Calvo-Maroto et al. [139] studied the effect of diabetes duration and poor glycemic control on the endothelial cell population: they found that the longer the

Table 2: Summary of "in vivo" studies about endothelial status in diabetic patients compared with healthy controls.

Author, year	Type of study	Study groups	Technology	Parameters	Results
Shenoy, 2009 [131]	Case-control Prospective	110 diabetic patients (110 eyes) 27 T1D 83 T2D 110 controls (110 eyes)	NIDEK® confoscan 2.	ECD, coefficient of variability of cell size of cells showing polymegathism, percentage of hexagonal cells showing pleomorphism.	(i) ECD in eyes was negatively associated with the diabetes status. (ii) The coefficient of variability in endothelial cells with polymegathism was 12 (8 to 16) more among eyes of diabetic patients than that of controls. (iii) The corneal endothelial cells with pleomorphism were 9% less in controls compared to the diabetic subjects.
Módis, 2010 [132]	Case-control Prospective	21 insulin-dependent T1D patients (41 eyes) 30 patients with non-insulin-dependent T2D (59 eyes). Control group 1 (22 patients, 40 eyes). Age-matched normal subjects with T1D group) Control group 2 (30 patients, 60 eyes). Age-matched normal subjects with T2D group)	Wide-field contact specular microscope (Tomey EM-1000, Tokyo, Japan)	ECD, mean cell area, CV, CCT, IOP	**T1D** (i) ECD decreased in T1D in comparison with controls. (ii) CCT thicker in T1D in comparison with controls. HbA1c level was inversely correlated with the ECD and correlated with the mean endothelial cell area. (iii) Positive correlation between glucose level and ECD, endothelial cell area and CCT. (iv) Negative correlation between ECD and duration of the disease/insulin therapy. **T2D** (i) No differences were found in the evaluated values compared to controls. No correlations were founded
Urban, 2013 [133]	Case-control Prospective	123 children and adolescents with T1D (123 eyes) 124 controls (124 eyes)	Topcon SP-2000P endothelial microscope.	ECD and CCT	(i) ECD was lower in children-adolescent T1D compared to controls. (ii) CCT was higher in children-adolescent T1D compared to controls. (iii) There was no correlation between ECD and metabolic control, HbA1c level and plasma creatinine level. (iv) Correlation between ECD, CCT, and duration of diabetes was statistically significant.

Table 2: Continued.

Author, year	Type of study	Study groups	Technology	Parameters	Results
Storr-Paulsen, 2014 [134]	*Case-control Prospective*	*107 T2D 128 controls*	*SP 2000P; Topcon, Tokyo, Japan.*	*ECD, CV, hexagonality percentage and CCT*	*(i) No differences between groups. (ii) Higher HbA1c was associated with lower ECD. (iii) CCT increased in the T2D group.*
Leelawongtawun, 2015 [135]	*Case-control Prospective*	*90 diabetic patients (171 eyes) (i) 1 patient (two eyes) with severe NP-DR. (ii) 7 patients (11 eyes) with moderate NP-DR (iii) 13 patients (24 eyes) with mild NP-DR (iv) 71 patients (134 eyes) with no DR 90 controls (156 eyes).*	*Specular microscope (Confoscan4, Nidek)*	*ECD, percentage of polymegathism, and hexagonality percentage.*	*(i) No differences between diabetes and controls (ii) The over one year diabetic patients had a decreased percentage of hexagonal cell compared to controls. (iii) The over two years diabetic patients had a decreased percentage of hexagonal cell and an increased percentage of polymegathism compared to controls.*
Calvo-maroto, 2015 [13]	Retrospective	77 noninsulin T2D (77 eyes): (i) Short-term diabetic subjects (recently diagnosed,<1 year since diagnosis) (ii) Long-term diabetic subjects (diagnosed and treated for 10 years or more) s80 controls (80 eyes)	Topcon SP-3000P noncontact specular microscope	CCT, ECD	(i) CCT higher in long-term diabetic patients when compared with short-term diabetic patients and controls. (ii) ECD lower in long-term diabetic patients when compared with short-term diabetic patients and controls.
Szalai, 2016 [12]	Case-control Prospective	28 T1D (28 eyes) 18 with DR 10 without DR 17 age-matched controls (17 eyes)	Corneal confocal microscopy with Heidelberg Retina Tomograph III Rostock Cornea Module (HRT III RCM, Heidelberg Engineering GmbH, Heidelberg, Germany)	ECD Other (epithelial, stromal density. Subbasal nerve morphology)	ECD was lower in T1D with and without DR compared to controls.
Anbar, 2016 [136]	Case-control Prospective	80 T1D children (160 eyes) 40 controls (80 eyes)	Noncontact specular microscope (Topcon SP-1P, Tokyo, Japan).	CCT, ECD, polymegathism, and pleomorphism	(i) CCT higher in the T1D group. (ii) ECD lower in the T1D group. (iii) Percentage of hexagonality lower in the T1D group. Polymegathism higher in the T1D group. (iv) All changes are correlated only with the duration of diabetes
Leelawongtawun, 2016 [137]	Case-control Prospective	148 diabetes (271 eyes). Divided based on diabetes duration (i) Below 5 years (ii) 5 to 10 years (iii) Over 10 years (iv) 46 controls (82 eyes)	Specular microscope (Confoscan4 (CS4), Nidek)	ECD, percentage of polymegathism and hexagonality percentage	(i) ECD was lower in all diabetes groups compared to controls. (ii) In all groups of diabetes, the polymegathism percentage was more than while the hexagonality percentage was less than controls. (iii) There were no differences in all endothelial parameters between 3 groups of diabetes.

Table 2: Continued.

Author, year	Type of study	Study groups	Technology	Parameters	Results
Galgauskas, 2016 [77]	Case-control Prospective	62 T2D (123 eyes): (i) 22 (17.9%) eyes with DR (ii) 10 (8.1%) eyes with macular edema 65 controls (120 eyes)	Noncontact specular microscope (SP-9000; Konan Medical Inc., Hyogo, Japan)	CCT, ECD, average size, hexagonality percentage and polymegathism	(i) ECD lower in diabetes than in controls. (ii) CCT higher in diabetes than in controls. (iii) Hemoglobin A1C and the duration of diabetes not associated with any of the examined parameters
El-agamy, 2017 [79]	Case-control Prospective	57 T2D (57 eyes): 36 eyes without DR 14 eyes with NP-DR 7 eyes with P-DR. 45 controls (45 eyes)	EM-3000 Specular Microscope	CCT, ECD, CV and hexagonality percentage	(i) ECD lower in diabetes than in controls. (ii) CV higher in diabetes.
Islam, 2017 [138]	Case-control Prospective	149 diabetes (149 eyes) (i) 52 T1D (ii) I97 T2D 149 controls(149 eyes)	SP–3000P, Topcon Corporation, Japan	ECD, average cell size, CV and hexagonality percentage	(i) ECD lower in the diabetes group Diabetes longer than 10 years had significantly lower ECD and larger average size. (ii) Diabetes duration was correlated with ECD, polymegethism and hexagonality
Qu, 2017 [13]	*Case-control Prospective*	*87 T2D (87 eyes): (i) 48 eyes without cornea fluorescein staining (ii) 39 eyes with cornea fluorescein staining 51 controls (51 eyes)*	*Keratograph 5M (K5M; OCULUS Optikgerate GmbH, Wetzlar, Germany)*	*Basal epithelial cell density, subbasal nerve plexus density, langerhans cell density and ECD.*	*No differences in ECD between groups*

T1D: type 1 diabetes; T2D: type 2 diabetes; DR: diabetic retinopathy; NP-DR: nonproliferative diabetic retinopathy; P-DR: proliferative diabetic retinopathy; ECD: endothelial cell density; CV: coefficient of variation of cell area; CCT: central corneal thickness; IOP: intraocular pressure.

evolution time of diabetes, the greater the loss of endothelial cells; this could be the reason why we find more differences in T1D patients, who are generally of a younger age at disease onset and usually present a longer duration of diabetes evolution. Islam et al. [138], Anbar et al. [136], and Urban et al. [133] also found this correlation between diabetes duration and ECD.

According to Storr-Paulsen et al. [134], and although they did not find statistically significant differences between groups with respect to ECD, higher glycated hemoglobin A1C levels were associated with lower ECD. Similar findings were described by Módis et al. [132] in T1D patients. Therefore, we can conclude that patients with longer disease evolution times and with poor metabolic control are those with higher endothelial loss.

Regarding endothelial characteristics, diabetes patients seem to have higher rates of polymegathism and lower percentages of hexagonality (higher polymorphism) [79, 131, 136, 137]. Moreover, Anbar et al. [136] and Islam et al. [138] found a significant correlation between the duration of diabetes and pleomorphism and polymegathism, supporting the idea that the longer the disease evolution, the more the endothelial alteration.

Another indicator of endothelial cell dysfunction, along with ECD, pleomorphism, and polymorphism, is CCT. The healthy cornea stays in a state of dehydration, as endothelial cell Na^+/K^+ ATPase and tight junctions are responsible for limiting the entrance of aqueous humor into the stroma [140]. When there is a substantial endothelial loss, the decrease in the number of tight junctions between cells allows more fluid to enter the stroma, favoring stromal rehydration with increased CCT that can lead to a loss of corneal transparency. Several authors have reported higher CCT in T1D [12, 133, 136] and T2D [77, 134] patients compared to controls, and Calvo-Maroto et al. [139] reported higher CCT in long-term T2D patients (diagnosed and treated for ten years or more) when compared with short-term T2D patients and controls.

Endothelial changes in the diabetic cornea can alter their function. Abnormal morphology of the corneal endothelial cells combined with increased CCT is an indicator of alterations of endothelial pump function, which can lead the cornea to a greater risk of decompensation following surgical trauma. Thus, a complete endothelial examination is important before ophthalmological procedures such as cataract surgery, since it is associated with an endothelial loss [141, 142].

5.1. In Vitro Studies. In vitro studies carried out over the last ten years with respect to the effect of diabetes on the corneal endothelium are summarized in Table 3. The findings in these donor tissue banks studies support the data observed in in vivo studies. Chocron et al. [148] and Liaboe et al. [145] reported lower levels of ECD in diabetes patients when compared to controls. Chen et al. [147] described this endothelial loss only in patients between 21 and 60 years; subjects above this age did not have statistically significant differences when compared to healthy controls. Moreover, Kwon et al. [143] report that age, previous cataract surgery, and diabetes were found to be the most important risk factors for deficient donor quality with respect to ECD.

Schwarz et al. [144] designed a method to assess differences in endothelium/Descemet membrane complex adhesion strength from stroma between diabetic and non-diabetic donor corneas. They did not find differences in ECD, hexagonality, or coefficient of variation of cell area between diabetes patients and controls; nevertheless, they observed greater resistance in diabetes patients for the separation between the endothelium/Descemet complex and the stroma.

There are two publications that analyze mitochondrial functioning in the endothelium of diabetes patients. Aldrich et al. [146] report that endothelial cells from insulin-dependent diabetes patients with medical complications had variations in their mitochondrial configuration, notable Golgi bodies associated with numerous vesicles, collection of lysosomal bodies/autophagosomes, and focal production of abnormal long-spacing collagen. Skeie et al. [149] found a decrease in mitochondrial proteins in corneas taken from patients with insulin-dependent diabetes when compared to those from patients with non-insulin-dependent diabetes. They suggest that proteins implicated in mitochondrial dysfunction decrease to a greater extent as diabetes progresses to insulin dependence, indicating that mitochondrial changes may be linked to diabetes insulin therapy itself or disease conditions at the time of transition to insulin therapy.

6. New Therapeutic Perspectives

In the past decade, certain therapies to treat specific corneal disorders in diabetes patients have been investigated. On one hand, these patients can benefit from the available symptomatic treatment options, such as artificial tear eye drops, topical anti-inflammatory drugs [150] (NSAIDs, steroids, and cyclosporine A), contact lenses [9], autologous serum, or platelet-rich plasma [4, 151]. It is also known that a strict metabolic control of blood glucose levels is important for prevention and treatment of ocular surface alterations in patients with diabetes [9]. On the other hand, new specific therapies for diabetic keratopathy and neuropathy are being investigated, even though they are in an experimental phase. Local therapy with substance P and IGF-1 has been shown to be effective in the treatment of diabetic keratopathy [152, 153], but more studies are needed to determine its effects on other ocular structures before its use can be recommended. There have also been studies that assess the effectiveness of substances such as aldose reductase inhibitor [154], the anti-inflammatory and healing agent TB4 [155], topical NGF [156], resolvin D [157], oral nicergoline [158], and antioxidants such as carnosine and β-carotene [159]. However, most of the suggested therapies have been investigated in animal models. A promising agent that has shown efficacy in several animal studies is naltrexone, an opioid antagonist which blocks opioid-receptor binding, thereby accelerating DNA synthesis [9]. In diabetes, there is an inhibition of cell proliferation due to the production of excessive opioid

Table 3: Summary of "in vitro" studies of the effect of diabetes on the corneal endothelium.

Author, year	Type of study	Study groups	Technology	Parameters	Results
Kwon, 2016 [143]	Descriptive	18,665 donors (34,234 corneas)	Specular microscopy (Konan Cell Chek EB-10; Konan Medical, Hyogo, Japan)	(i) Sex, age, race, surgery, disease (hypertension, diabetes, glaucoma, depression, dementia, Parkinson, hyperthyroidism and hypothyroidism) and habits (smokers/ nonsmokers) (ii) All independent variables were divided into 2 groups: (1) ECD>2000 cels/mm^2 (2) ECD<2000 cels/mm^2	(i) ECD decreased with age. (ii) The average ECD of African American donors was higher than those of white or Hispanic donors. (iii) A history of diabetes and ocular surgery were associated with a lower ECD. (iv) Age, history of cataract surgery and diabetes were found to be the greatest risk factors for inadequate donor quality with respect to ECD.
Schwarz, 2016 [144]	Case-control	22 donors (27 corneas): (i) Nondiabetes (9 corneas, 8 donors) (ii) Diabetes without evidence of advanced disease (8 corneas, 7 donors) (iii) Diabetes with evidence of advanced disease (10 corneas, 7 donors).	(i) Specular microscopy (technology not specified) (ii) The adhesion strength of endothelium-descemet membrane complex to the posterior stroma was measured by an own method developed by the investigators (see article).	(i) ECD, hexagonality, and CV. (ii) Variables obtained from mechanical peel testing were: (1) Endothelium-descemet membrane complex elastic peel tension (TE) (2) Elastic stiffness (SE) (3) Average delamination tension (TD), and maximum tension (TMAX)	(i) The three groups did not differ in ECD, hexagonality, and CV. (ii) Diabetes with evidence of advanced disease had values for TE, TD, and TMAX greater than nondiabetes and diabetes without evidence of advanced disease corneas.
Liaboe, 2017 [145]	Retrospective case-controls	2112 donors (4185 corneas) divided in 4 groups: (i) Nondiabetes(2636 corneas) (ii) NID-diabetes (847 corneas) (iii) ID-diabetes without medical complications due to diabetes (471 corneas) (iv) I-diabetes with medical complications due to diabetes (231 corneas).	Noncontact specular microscopy (KeratoAnalyzer EKA-10; Konan Medical USA, Irvine, CA)	Donor age, death to preservation time, ECD, hexagonality, and CV.	(i) I-diabetes with medical complications due to diabetes corneas showed a significant reduction in mean ECD compared with nondiabetic and NI-diabetes. (ii) There were no significant differences in endothelial cell hexagonality or coefficient of variation among the 4 groups.

Table 3: Continued.

Author, year	Type of study	Study groups	Technology	Parameters	Results
Aldrich, 2017 [146]	Case-control	159 donors (229 corneas) all of them with ECD> 2000 cells/mm^2. Divided in 4 groups: (i) Nondiabetes (ii) NID-diabetes (iii) ID-diabetes without medical complications due to diabetes (iv) ID-diabetes with medical complications due to diabetes	(i) Noncontact specular microscopy (KeratoAnalyzer EKA-10; Konan Medical USA, Irvine, CA, USA) (ii) Transmission electron microscopes (EM 906E; Carl Zeiss Microscopy, Oberkochen, Germany)	(i) ECD, hexagonality, and CV. (ii) Qualitative and quantitative ultrastructural changes in corneal endothelial cells quantified with transmission electron microscope: (iii) Number of mitochondria per μm^2, surface area per mitochondria in μm^2, and total mitochondrial surface area per 20 μm^2 field of view.	(i) ID-diabetes with medical complications due to diabetes displayed the lowest spare respiratory values compared to all other groups. (ii) The remaining mitochondrial respiration and glycolysis metrics did not differ significantly among groups. (iii) Compared to nondiabetes, the endothelium from ID-diabetes with medical complications due to diabetes had alterations in mitochondrial morphology, pronounced Golgi bodies associated with abundant vesicles, accumulation of lysosomal bodies/ autophagosomes, and focal production of abnormal long-spacing collagen.
Chen, 2017 [147]	Case-control	(i) 20,026 nondiabetes donor eyes (ii) 13,617 diabetes donor eyes	Specular microscope (Konan EB-10; Konan, Hyogo, Japan).	ECD	Amongst phakic donors, diabetic ECD was lower in the middle aged subgroups, between 21 and 40 years and between 41 and 60 years. There was no difference in ECD for phakic corneas from the subset aged 61 years or older.
Chocron, 2018 [148]	Retrospective case-control	17056 donors: (i) Diabetes (4766 patients): (ii) Metformin consumers (iii) Nonmetformin consumers (iv) Controls (12290 patients)	Specular microscopy (Konan Cell Check EB-10; Konan, Hyogo, Japan)	Age, sex, race, medical history, medication list at the time of death, and ECD.	(i) ECD was lower in patients with diabetes. (ii) ECD was not associated with metformin use in patients with diabetes. (iii) Metformin use was significantly associated with lower ECD among patients with glaucoma.

Table 3: Continued.

Author, year	Type of study	Study groups	Technology	Parameters	Results
Skeie, 2018 [149]	Case-control	19 donors: (i) 4 nondiabetes (ii) 10 nonadvanced diabetes (without or with history of home insulin use) (iii) 5 advanced diabetes with medical complications due to diabetes (history of home insulin use and end-organ damage specifically noted in the medical history)	Multidimensional protein identification technology mass spectrometry	Corneal endothelial cell layer and descemet membrane proteome characterization	(i) Decrease in relative protein abundance in insulin-dependent samples (nonadvanced diabetes insulin-dependent and advanced diabetes) compared to non-insulin-dependent samples (nondiabetes and nonadvanced diabetes without insulin use). (ii) Comparing the nonadvanced diabetes insulin-dependent and advanced diabetes groups, mitochondria protein levels appear to increase as the disease progresses.

NID-diabetes: non-insulin-dependent diabetes mellitus; ID-diabetes: insulin-dependent diabetes mellitus. ECD: endothelial cell density; CV: coefficient of variation of cell area.

growth factors. The topical application of naltrexone has been shown to be useful both for corneal regeneration and tears production, improving the corneal sensitivity in T1 and T2 diabetic animal models [45, 160]. In addition, there are promising novel therapeutic approaches that include gene [23, 24, 41] and stem cells therapies [4, 44]; nevertheless, at the moment, they are in preclinical development. In the near future, we can expect some advances in the prevention and management of corneal disorders associated with diabetes, possibly from a multidisciplinary point of view.

In conclusion, different corneal components (epithelium, stroma, nerves, and endothelium) suffer specific complications of diabetes. The development of new non-invasive diagnostic technologies has provided a better understanding of corneal tissue changes related to diabetes. The published literature sheds light on the potential utility of the biomechanical corneal properties to improve our understanding of the mechanical behavior of this complex tissue in diabetes patients. However, the literature shows controversial results in relevant areas such as CH and its impact on IOP measurement. New technologies are showing promise in consolidating the utility of the biomechanical corneal properties as a clinical tool and a relevant field for the future improvement of diagnosis of diabetes and control of the disease.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Scheimpflug Camera and Swept-Source Optical Coherence Tomography in Pachymetry Evaluation of Diabetic Patients

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Aim. The comparative analysis of the central and peripheral corneal thicknesses using two different imaging systems: Scheimpflug camera and swept-source OCT was performed to investigate the differences in corneal thickness analysis in diabetic patients. *Materials and Methods*. The study group consisted of the 147 eyes of 107 diabetic patients who were examined and compared with 138 eyes of 89 nondiabetic cataract patients. The inclusion criteria for the study group was diabetes mellitus type II identified no less than 10 years ago, with NPDR not requiring prior laser treatment. The control group was recruited from nondiabetic patients. Measurements were obtained on the Pentacam Scheimpflug imaging system and Casia swept-source OCT. All study parameters from anterior chamber images were processed for five different zones, the central zone and four peripherals—superior, inferior, nasal, and temporal. A fit zone diameter of 4 mm was applied for both instruments. *Results*. The Pentacam system overestimated corneal measurements in the DM group when compared with the Casia OCT in superior corneal zone ($p = 0.04$), inferior corneal zone ($p = 0.02$), nasal corneal zone ($p < 0.001$), and temporal corneal zone ($p = 0.01$). In the control group, there were also statistically significant differences between the Pentacam and Casia OCT measured values in inferior corneal zone ($p = 0.001$), nasal corneal zone ($p = 0.04$), and temporal corneal zone ($p < 0.001$). *Conclusion*. Scheimpflug camera pachymetry measurements showed statistically higher CCT values when compared with swept-source OCT measurements.

1. Introduction

Diabetes mellitus (DM), one of the most common metabolic disorders worldwide, is associated with many ocular complications. First of all, diabetic retinopathy (DR) affects the retinal vessels and is divided into two main groups, namely, nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) [1, 2]. Patients with DM are also predisposed to damage of all layers of the cornea. Morphological changes of the cornea include the corneal endothelium playing a vital role in keeping the stroma dehydrated [3]. The damage manifests in decreased endothelial cell density, polymorphism, and polymegathism [4–6]. Also, the reduced density of basal epithelial cells, associated with haemoglobin A1c and advanced glycation end products, plays important role in disorders of the corneal surface [7, 8]. The previously mentioned disorders lead to endothelial dysfunction and differences in corneal thickness [4, 5, 7–9]. It may lead to changes in the refractive errors and corneal transparency. DM is responsible for damage of the pericytes and vascular endothelium causing reduced blood supply to Schwann cells or neurons [3, 10].

Neurotrophic loss of corneal sensation leads to reduced tear production and its consequences like dryness of the eye, punctate keratitis, persistent epithelial defects, or impaired corneal sensitivity, so-called diabetic keratopathy [3, 10, 11]. Structural and functional changes in corneas contribute to increased surgical risk, complications, and prolonged corneal healing [1, 3, 5, 10].

Other ophthalmic manifestations of DM include changes in lens transparency and premature cataract development, altered pharmacological mydriasis, orbital and lid features like cranial nerve palsies, chalazia, xanthelasma, and cellulitis, and conjunctival abnormalities comprising pterygia, pinguecula, tortuosity, and dilation of conjunctival vessels [11, 12].

The imaging of anterior chamber structures should be estimated with objective qualitative and quantitative methods. Corneal thickness can be measured using different devices, such as Scheimpflug camera imaging, optical pachymetry, confocal microscopy, ultrasound biomicroscopy, scanning slit topography, scanning peripheral anterior chamber depth analyser, time-domain optical coherence tomography (OCT), or ultrasound pachymetry [13–16]. Until recently, ultrasound was considered the gold standard in pachymetry, and most ophthalmologists are familiar with this device. However, despite common accessibility, this method has limitations. It is not a global pachymetry measurement, as only specific points can be measured, and it requires local anaesthesia and aseptic precautions [13, 14].

The aim of this study was the comparative analysis of the central and peripheral corneal thicknesses using two different imaging systems of measurement, the Pentacam Scheimpflug camera and Casia swept-source OCT, to investigate the effect of DM type II with DR not requiring photocoagulation on corneal thickness.

2. Materials and Methods

The study was performed at the Ophthalmology Department of Saint Barbara Hospital, Trauma Centre, Sosnowiec, Poland. The study was conducted under tenets of the Declaration of Helsinki. All patients signed an informed consent form before ophthalmic examination and surgical procedures.

2.1. Participants. Patients from the study and control groups were recruited from cataract patients operated on between January 1, 2018, and September 30, 2018. Basic information was collected from all patients including age, sex, medical history, and duration of diabetes mellitus. Complete ophthalmic examinations including preoperative best-corrected Snellen distance visual acuity, intraocular pressure (IOP) measurement using Goldmann applanation tonometry, slit-lamp biomicroscopy, and fundus examination with a dilated pupil were performed. Randomisation of groups was done according to inclusion/exclusion criteria. The inclusion criteria for the study group was DM type II identified no less than 10 years ago, with NPDR not requiring prior laser photocoagulation. The control group was recruited from nondiabetic cataract patients. The exclusion criteria for both patient groups were all systemic and ophthalmic conditions likely to affect the corneal state and thickness. This included corneal pathologies such as scars and haze, degenerations and dystrophies, pseudoexfoliation syndrome, previous ocular surgeries (mainly corneal refractive surgery) or ocular trauma, ocular hypertension or glaucoma, uveitis, contact lens wearers, cornea-depending refractive error (±4.0 spherical dioptres and ±2.5 cylindrical dioptres), usage of topical medication which may affect the ocular surface and corneal condition (mainly medications with preservatives), and systemic diseases with ocular involvement, like autoimmune or inflammatory diseases. Randomisation was done during routine admissions to the hospital.

The study group consisted of the 147 eyes of 107 diabetic patients who were examined and compared with 138 eyes of nondiabetic 89 cataract patients. All measurements submitted for the study were obtained prior to cataract surgery.

Measurement of corneal thickness was determined by one operator using two different imaging systems, the Pentacam Scheimpflug imaging system and Casia swept-source OCT. All study parameters from anterior chamber images were processed for five different zones, the central zone and four peripherals—superior, inferior, nasal, and temporal. A fit zone diameter of 4 mm was applied for both instruments. Two consecutive measurements were obtained for each eye of each patient for each device.

2.2. Instruments. The Pentacam Scheimpflug imaging system (Pentacam HR, Oculus, Wetzlar, Germany) uses rotating cameras to reconstruct the three-dimensional structure of the cornea from two-dimensional optical sections, which provide sharp images for detailed analysis from the anterior corneal surface through the posterior aspect of the crystalline lens. It uses a 475 nm wavelength blue light-emitting diode (LED) to provide anterior and posterior surface topography of the cornea, pachymetry, anterior chamber angle, depth, and volume data as well as crystalline lens analysis (densitometry). The instrument-based software allows automatic analysis of various anterior segment parameters and takes 25 images per measurement within two seconds. It captures 100 slit images with a slip depth of 14.0 mm in 2 s by rotating along the optical axis from 0° to 360°. Central corneal thickness (CCT) is measured as the difference between anterior and posterior elevations in the central cornea [13, 15, 16].

Swept-source OCT (Casia SS-1000, Tomey, Nagoya, Japan) is a swept-source anterior segment OCT that uses a wavelength of 1310 nm and performs measurements with a speed of 30,000 axial scans per second. In the corneal map mode, each 3D image consists of 16 B-scans and 512 A-lines, and in the anterior segment mode, each 3D scan contains 128 B-scans and 512 A-scans. Total scan duration is 0.3 s for measurement of corneal thickness and corneal topography. The software automatically analyses the recorded images and provides various corneal maps, as well as a quantitative and qualitative anterior segment structure evaluation [17–19].

The axial resolution, offered by both noncontact devices, is 10 μm for Pentacam-Scheimpflug camera and 10 μm for CASIA OCT .

2.3. Statistical Analysis. The computer software XLSTAT-Biomed (Addinsoft SARL, France) was used for statistical analysis and to calculate means and standard deviation. The parameter values were compared between the control and DM groups using the Student's *t*-test or Mann–Whitney *U* test. In a Bland–Altman plot, the difference between measurements with different methods is plotted against their mean. The 95% limit of agreement (mean difference ± 1.96 standard deviation) provides the distance between measurements with 95% confidence. The Bland–Altman plot also shows proportional bias in the measurements, which is the relationship of the difference between measurements and the true value. A value less than 0.05 was considered statistically significant.

3. Results

Between January 1, 2018, and September 30, 2018, 107 diabetic patients (55 females and 52 males), with NPDR not requiring prior laser photocoagulation, and 89 nondiabetic patients (46 females and 43 males) underwent phacoemulsification surgery with in-the-bag intraocular lens implantation. The mean age of the study group was 71.85 ± 8.04 years (range 49–88 years old) and of the control group was 69.08 ± 9.13 years (range 45–84 years old). There was no statistically significant difference with respect to gender or age between the groups. In the DM group, the disease was recognized during routine glucose level tests, performed by GPs usually in every year.

Table 1 shows average pachymetry and the standard deviation of five different corneal zones measured by two different systems, the Pentacam Scheimpflug imaging system and CASIA swept-source OCT, in diabetic and control group patients, respectively.

The Pentacam overestimated corneal measurements in the DM group when compared with the Casia: superior corneal zone ($p = 0.04$), inferior corneal zone ($p = 0.02$), nasal corneal zone ($p < 0.001$), and temporal corneal zone ($p = 0.01$). In the control group, there were also statistically significant differences between the Pentacam and Casia measured values: inferior corneal zone ($p = 0.001$), nasal corneal zone ($p = 0.04$), and temporal corneal zone ($p < 0.001$).

Central and nasal corneal zone thicknesses measured with both methods had a statistically significant difference. On the contrary, measurements of the temporal corneal zone with both scanning methods had no statistically significant values. Statistically significant differences were also observed between Pentacam measurements for the inferior corneal zone and between Casia measurements for the superior corneal zone.

The Bland–Altman plot illustrates the level of agreement between the two instruments for each scan type, as well as the mean of the difference between evaluations generated by the two instruments, Pentacam and Casia, in the study group (Figure 1).

4. Discussion

The term "diabetic eye" is mostly thought to refer to the retinal, not corneal, pathology. Different corneal imaging systems are used to identify corneal pathologies and their progression. An accurate corneal thickness evaluation is crucial for IOP measurement prior to corneal and many other types of ocular surgery [9, 14, 20–23].

In our study, we compared two noncontact corneal thickness measurement devices, the Pentacam Scheimpflug imaging system and CASIA swept-source OCT, in diabetic patients with NPDR not requiring prior laser photocoagulation and with nondiabetic cataract patients.

Different studies postulate the influence of hyperglycaemia on endothelial dysfunction with consistent stromal hydration and swelling of the cornea [2, 5, 8, 24].

The results obtained by Elflein et al. [25], like Kotecha et al. [26], show no association between CCT and diabetes. Nevertheless, that the average CCT is significantly higher in diabetic versus nondiabetic patients is commonly underlined by different researchers. However, the interpretation of those results should include the type and duration of DM, type of retinal changes, and the method of treatment [6, 22, 24, 27–29]. The results of Senćanić et al. [29] show no significant difference in CCT between diabetic patients without diabetic retinopathy and NPDR, but a statistically significant difference in CCT between patients without diabetic retinopathy and PDR. The highest mean CCT values in this study were recorded in the PDR patients, followed by the NPDR group.

Qu et al. [7] divided the cornea into five zones (central, superior, inferior, nasal, and temporal), as in the current study. They evaluated parameters affecting corneal thickness—endothelial cells, basement epithelial cells, and subbasal nerve plexus. The central endothelial cell density was not significantly different in the diabetic patients and healthy controls. This is contrary to many other findings [5, 6, 24, 30].

Hashemi et al. [31] compared central and peripheral corneal thicknesses between diabetic and nondiabetic patients during a five-year period using the Pentacam. The diabetic group showed less reduction in all corneal thickness zones than the nondiabetic group.

Sanchis-Gimeno et al. [21] evaluated differences in central and four midperipheral corneal thicknesses between type II diabetic patients and nondiabetic patients using the Orbscan Topography System II. Our results for the study group, both from the Pentacam and Casia, are compatible with reference to those authors' findings. Our control group results are partly different only in the Pentacam measurement group. The superior and nasal corneal thicknesses are comparable, and the temporal corneal zone has a greater value than the inferior.

But, an exact comparison of corneal central and peripheral pachymetry values using different measuring methods in diabetic individuals was not possible due to a lack of data in the reviewed literature.

Table 1: Mean pachymetry values by corneal regions for the study groups.

		Value (μm) and region				
Technique	Group	Central	Superior	Inferior	Nasal	Temporal
Pentacam	DM	552 ± 23	577 ± 26	564 ± 27	575 ± 26	563 ± 25
	Control	543 ± 26	578 ± 20	559 ± 22	578 ± 21	561 ± 22
	p value	0.005	0.865	0.018	0.01	0.2
Casia OCT	DM	548 ± 22	567 ± 27	552 ± 28	562 ± 25	553 ± 25
	Control	541 ± 29	573 ± 23	551 ± 22	572 ± 20	549 ± 20
	p value	0.041	0.031	0.431	<0.001	0.119

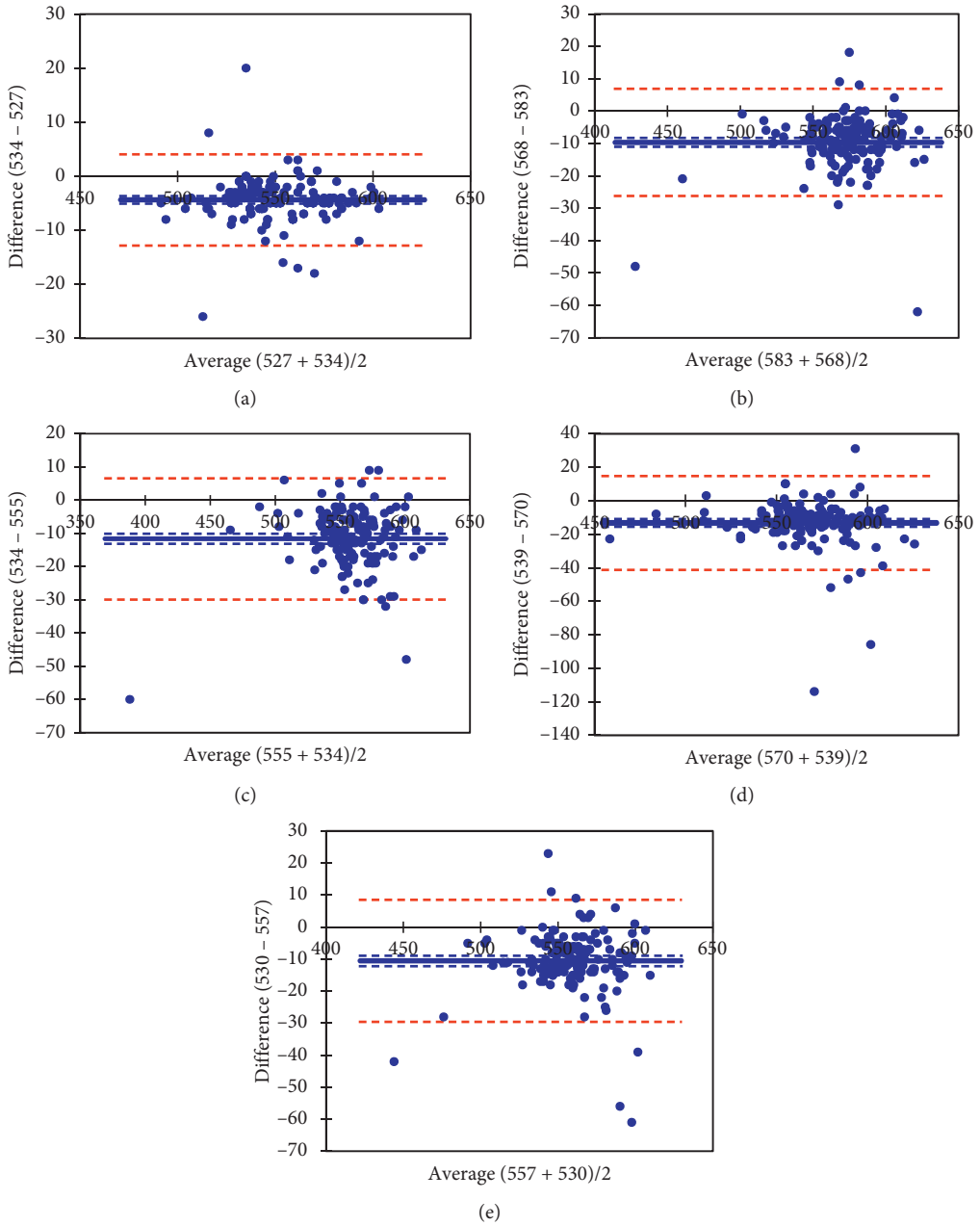

Figure 1: Bland–Altman plots showing agreement between Pentacam and Casia pachymetry measurements in five different corneal zones for the study group. (a) Central corneal thickness (bias = −4.37 ± 4.29); (b) superior corneal zone (bias = −9.66 ± 8.49); (c) inferior corneal zone (bias = −11.61 ± 9.29); (d) temporal corneal zone (bias = −13.18 ± 14.3); (e) nasal corneal zone (bias = −10.52 ± 9.73).

The differences in values of measured parameters are also dependent on measuring methods and devices. Ultrasonic pachymetry, considered the gold standard for pachymetry, carries the risk of development of corneal epithelial defects and transmission of infection. Contemporary noncontact systems offer repeatability and a range of quantitative and qualitative information. Pentacam Scheimpflug differs in many aspects when compared with Casia. However, scan quality and axial resolution do not make this device worse in corneal thickness assessment.

In our study, Pentacam pachymetry measurements indicated statistically higher CCT values when compared with Casia measurements. These measurements are comparable with results reported in other studies [13, 18, 32]. Otherwise, the results of the Choo et al.'s [4] study revealed that however endothelial cell density is reduced and polymorphism and polymegathism are increased, CCT is unaffected.

There are several limitations of our study. Diabetes mellitus is not a homogenous disease, it has different stages, ocular and systemic complications and associations; therefore, the need for further studies cannot be overemphasised. Each factor should be taken into account when measuring corneal anatomical and biomechanical parameters.

In conclusion, the results of our study show the CCT in patients diagnosed no less than 10 years ago, with NPDR not requiring prior laser photocoagulation, is significantly higher than that in healthy individuals. This finding should be taken into account when measuring the IOP, diagnosing intraocular hypertension or glaucoma, or before any ocular surgery. Routine CCT measurement in diabetic patients may also be beneficial in the evaluation and treatment of diabetic keratopathy as well as corneal neuropathy.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Structural and Biomechanical Corneal Differences between Type 2 Diabetic and Nondiabetic Patients

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Purpose. To analyze and compare corneal structural and biomechanical properties, characterized by corneal hysteresis (CH) and resistance factor (CRF), between patients with and without type 2 diabetes mellitus (DM), and determine the main ocular variables that influence them. *Methods*. Sixty diabetic and 48 age- and sex-matched non-DM patients were enrolled in this cross-sectional study. The DM group was analyzed according to DM duration ($<$or$\geq$10 years), HbA1c levels ($<$or$\geq$7%), and presence of retinopathy. CH and CRF were evaluated using the Ocular Response Analyzer® (ORA). Central corneal thickness (CCT) was determined by Scheimpflug tomography (Pentacam® HR). Intraocular pressure was obtained with ORA (IOPcc) and Goldmann applanation tonometry (IOP-GAT). Univariate and multivariate linear regression analyses were performed to evaluate the relationship between demographical, clinical, and ocular variables with the biomechanical properties. *Results*. There were no statistically significant differences in the CH and the CRF between DM and non-DM groups ($p = 0.637$ and $p = 0.439$, respectively). Also, there was no statistical difference between groups for the CCT, IOPcc, or IOP-GAT. Multivariate linear regression analysis showed that CH was positively associated with CCT ($p < 0.001$) and negatively associated with IOPcc ($p < 0.001$), while CRF was positively associated with CCT ($p < 0.001$) and IOPcc ($p = 0.014$). *Conclusion*. The CCT and IOPcc were found to be the main parameters that affect corneal biomechanical properties both in diabetics and controls. In this study, there was no significant effect of DM type 2 on corneal biomechanics.

1. Introduction

In the last decade, there has been a growing interest in the study of corneal biomechanics suggesting that the cornea acts as a viscoelastic structure that might be influenced by ocular and systemic conditions [1].

In clinical practice, corneal biomechanical properties can be easily and accurately [2, 3] estimated using the Ocular Response Analyzer® (ORA). It evaluates corneal deformation response though a calibrated air puff and calculates corneal hysteresis (CH) and resistance factor (CRF) [4]. CH predominantly reflects the viscoelastic response of the cornea to an applied force defined by a specific air-pressure curve [5], whereas the CRF provides information on overall resistance of the cornea to deformation. It is important to note that CH and CRF are not directly related, and alterations in tissue structure can lead to independent changes in both parameters [6]. The ORA also provides a biomechanically adjusted estimate of intraocular pressure (IOPcc) that is less affected by corneal thickness or curvature [7].

Recent evidence has shown that the central corneal thickness (CCT) only accounts for a small fraction of the variance in IOP when compared to the biomechanical properties of the cornea. In fact, CH was found to be more strongly associated with glaucoma presence, risk of progression, and effectiveness of glaucoma treatments than CCT [8].

The relationship between corneal morphological changes and elevated plasma glucose concentrations in diabetes mellitus (DM) has been extensively reviewed. Most importantly, the presence of hyperglycemic states can cause a nonenzymatic glycosylation of collagen, proteoglycans, and glycosaminoglycans (Maillard reaction) that results in increased corneal stiffening [9].

Clinical investigations have shown that adult diabetic subjects may have altered corneal biomechanics [10–18]; however, this relationship is far from being clarified. For example, CH was reported to be greater in DM patients compared to non-DM subjects [11–15], while others reported no differences or significantly inferior values [16, 17, 19]. One reason for this is that different studies had different definitions of DM (by interview or glycated hemoglobin A1c (HbA1c) levels), selection criteria (DM type and severity), and designs (control of confounding factors such as IOP and CCT), resulting in contradictory results. Also, no studies have addressed eventual corneal biomechanical associations to different stages of diabetic retinopathy (DR).

The purpose of this study is to evaluate the differences in corneal structural and biomechanical properties between patients with and without type 2 DM. In addition, it aims to determine the main ocular variables that influence the corneal biomechanics.

2. Methods

2.1. Subjects. A cross-sectional observational study was performed. Type 2 diabetic patients with different stages of DR and controls, aged 50 or older, were prospectively recruited from the Cataract and Refractive Surgery Unit of the Ophthalmology Department of Centro Hospitalar São João between September 2015 and March 2016. Medical records of all patients scheduled for monocular phacoemulsification cataract surgery were reviewed, and eligible patients were invited to participate. Informed consent was obtained from each participant before inclusion in the study. The study protocol adhered to the tenets of the Declaration of Helsinki and received Institutional Review Board approval.

The diagnosis of type 2 DM was based on the medical history, HbA1c levels ≥6.5%, and/or current use of antidiabetic medication [20]. Nondiabetic age- and sex-matched patients were used as controls. All study participants were Caucasian. The same exclusion criteria were used for both groups, and they included prior eye surgery (except for anti-VEGF agents or triamcinolone intravitreal injections < 120 days or laser photocoagulation < 90 days before surgery in the study eye of diabetics), any corneal, retinal, or optic nerve disease except DR (e.g., glaucoma, age-related macular degeneration, vascular occlusions, uveitis, and other chorioretinal diseases), mature cataracts (nuclear opacity grade greater than 3, from 1 (mild) to 4 (white/brown) severity grading system), Goldmann applanation tonometry (IOP-GAT) > 25 mmHg, pseudoexfoliation syndrome, current treatment with glucocorticoids, and ORA waveform score (WS) ≤ 3.5 [21]. Diabetic patients were excluded if they had uncontrolled complications of proliferative DR (e.g., current iris neovascularization, vitreous hemorrhage, or tractional retinal detachment). All patients with a serious illness or syndrome and any physical or mental problem that could hinder the examinations required for the study were also not included.

2.2. Study Protocol. All subjects underwent a standard examination which included a general anamnesis to obtain demographical and medical history (ocular and systemic). Before measurements, each participant was subjected to a complete ophthalmic evaluation performed in a standardized fashion by the same ophthalmologist. The grade of DR was assessed in all diabetic patients using 7 standard ETDRS fundus photographs [22].

The examinations were sequentially performed with the IOLMaster® 500 (software version 7.7) and then with the Pentacam® HR Scheimpflug tomographer (Pentacam HR version 6.08r19 with the software version 1.20r87). Measurements were repeated as necessary until high-quality images were obtained. Only good-quality examinations were accepted, defined as scans that passed the software's quality check.

Corneal biomechanical properties were assessed using the ORA (software version 1.1). After checking for a good alignment of the eye and the probe, a series of four good-quality measurements was performed on both eyes of each subject. For each eye, the measurement with the higher waveform score was used for the analysis, as recommended by the manufacturer.

All measurements were performed by an experienced operator (JB) in a darkened room between 1 and 7 pm, without cyclopegia, and the patients were told to blink immediately before each examination.

Following instillation of topical corneal anesthesia (oxybuprocaine hydrochloride 0.5% with fluorescein sodium 0.25%), the IOP was measured twice by a masked investigator (JEL) using the Goldmann applanation tonometer (GAT).

At the end of the visit, an experienced nurse evaluated all the individuals to record vital signs and collect blood samples, by venous puncture, for serum HbA1c analysis. These allowed the authors to evaluate the glycemic status of DM patients and disclose undetected diabetes in the non-DM group.

2.3. Devices

2.3.1. IOLMaster® 500 (Carl Zeiss Meditec, Jena, Germany). The IOLMaster is a partial coherence interferometer used for optical biometry. It measures the AL (mean of five measurements) through an infrared light (780 nm) and has been shown to have high intraobserver and interobserver reproducibility [23].

2.3.2. Pentacam® HR (Oculus, Wetzlar, Germany). The Pentacam uses a single 180-degree rotating Scheimpflug

camera and a monochromatic slit-light source (blue LED at 475 nm) combined with a static camera (for the correction of any eye movement) to generate a three-dimensional high-resolution (HR) image of the anterior segment. Anterior keratometry and apex paquimetry (CCT) have been shown to have excellent repeatability and reproducibility [24].

2.3.3. Ocular Response Analyzer® (Reichert Ophthalmic Instruments, New York, USA). The ORA is a noncontact tonometer that uses a calibrated air puff and infrared electro-optical system to measure the required force to flatten the cornea as the air pressure rises (force-in applanation, P1) and the force at which the cornea becomes flat again as the air pressure falls (force-out applanation, P2) [4]. It determines four basic parameters based on the 2 pressure measurements at applanation. The difference between P1 and P2 is called CH and represents the viscoelastic properties of the cornea. The average of P1 and P2 is called Goldmann-correlated IOP (IOPg). Through empirical investigation, 2 other parameters, calculated as a linear function of both applanation pressures, were defined: corneal resistance factor (CRF), which is supposed to be more correlated with CCT, and corneal-compensated IOP (IOPcc), which was designed to be similar before and after refractive surgery [5].

2.4. Sample Size Calculation. For a type I error of 0.05 and type II error of 0.20 (80% power), considering a mean difference of CH ≥ 1 mmHg to be significant and assuming the SD for the non-DM group of 1.7 mmHg [10, 16, 17], the minimal required sample size would be 46 subjects in each group. We included additional patients in the DM group in order to perform subgroup analysis.

2.5. Data and Statistical Analysis. Diabetic subjects were classified into subgroups according to DM duration (<10 and ≥10 years), HbA1c levels (<7.0 and ≥7.0%), and DR (absence or presence of DR). According to patient self-reports, smoking status was evaluated (never smokers and active/former smokers groups). Body mass index (BMI, in kg/m^2) was calculated as weight/height^2 using measured weight and height.

Statistical analysis was performed using the SPSS® statistical software (version 21.0 for Mac OS; SPSS Inc., Chicago, IL, USA). In the present study, only the fellow nonscheduled eye of each patient undergoing monocular cataract surgery was used for statistical analyses. The Kolmogorov–Smirnov test and normal probability plots were used to confirm the normal distribution of the data. Parametric or nonparametric tests were used for continuous variables comparison between the DM and non-DM groups, according to the normality of data. Chi^2 or Fisher's exact tests were performed for categorical variables comparison. Univariate and multivariate linear regression analyses, using generalized linear models, were performed to identify the potential demographical/clinical (age, gender, body mass index (BMI), DM duration, HbA1c levels, and smoke history) and ocular variables (AL, Km, CCT, IOPcc, and IOP-GAT) associated with CH and CRF. Statistical significance for all the analyses was set at a p value less than 0.05.

STROBE guidelines were followed for manuscript elaboration [25].

3. Results

Sixty diabetic patients and 48 nondiabetic controls were enrolled in the study. Demographic and clinical characteristics of the study population did not show any significant differences between groups, except for the levels of HbA1c (Table 1).

In the DM group, duration of DM was significantly associated with HbA1c levels ($p = 0.004$, chi^2 test) and severity of DR ($p = 0.014$, Fisher's exact test), as well as severity of DR and HbA1c levels ($p = 0.028$, Fisher's exact test).

3.1. Comparison of Ocular Parameters between DM and Non-DM Groups. There were no significant differences between groups for any of the studied variables IOP-GAT, IOPcc, CH, and CRF (Table 2).

3.2. Subgroup Analysis of Corneal Biomechanics in the DM Group

3.2.1. Duration of Diabetes. There were no statistically significant differences in the CH and the CRF between the DM group ≥10 years and <10 years ($p = 0.233$ and $p = 0.189$, respectively) (Table 3).

3.2.2. HbA1c Levels. There were no statistically significant differences in the CH and the CRF between the DM group with HbA1c ≥ 7% and HbA1c < 7% ($p = 0.507$ and $p = 0.228$, respectively) (Table 3).

3.2.3. DR Stage. There were no statistically significant differences in the CH and the CRF between DM groups with and without retinopathy ($p = 0.440$ and $p = 0.742$, respectively) (Table 3).

3.3. Factors Influencing the CH. Multivariate linear regression analysis showed that CH was positively associated with CCT ($p < 0.001$) and negatively associated with IOPcc ($p < 0.001$). In a "fixed model," the CH was found to significantly increase on average 0.02 mmHg for each increase of one micron of CCT, whereas it significantly decreased on average 0.21 mmHg for each increase of 1 mmHg of IOPcc (Table 4).

3.4. Factors Influencing the CRF. In multivariate linear regression analysis, CRF was positively associated with CCT ($p < 0.001$) and IOPcc ($p = 0.014$). In a "fixed model," the CH was found to significantly increase on average 0.02 mmHg for each increase of one micron of CCT, whereas

Table 1: Demographic and clinical characteristics of the study population.

	DM group ($n = 60$)	Non-DM group ($n = 48$)	p
Age (y)	72.38 ± 5.66	70.21 ± 6.45	0.065[1]
Female (n)	38 (63.3%)	30 (62.5%)	0.929[3]
Right eyes (n)	30 (50.0%)	19 (39.6%)	0.280[3]
BMI (kg/m^2)	27.91 ± 4.01	27.97 ± 5.01	0.943[1]
Smoking history (n)	14 (23.3%)	18 (37.5%)	0.109[3]
HbA1c levels (%)	7.02 ± 1.13	5.54 ± 0.35	<0.001*[2]
Duration of diabetes (y)	10.98 ± 8.03	n/a	n/a
DR stage (n)			
NPDR absent	42 (70.0%)		
NPDR mild-moderate	10 (16.7%)	n/a	n/a
NPDR severe-PDR	8 (13.3%)		
Oral antidiabetic agents (n)	56 (93%)	n/a	n/a
Insulin treatment (n)	15 (25%)	n/a	n/a

Data were derived from [1]independent samples t-test, [2]Mann–Whitney U-test, and [3]chi-squared test. Continuous variables are reported as mean ± standard deviation. * $p < 0.05$ represents statistical significance. DR, diabetic retinopathy; NPDR, nonproliferative DR; PDR, proliferative DR; n/a, not applicable; y, years.

Table 2: Ocular characteristics and ORA measurements of the study population.

	DM group ($n = 60$)	Non-DM group ($n = 48$)	p
Axial length (mm)	22.98 ± 0.94	22.91 ± 0.75	0.683[1]
Km (D)	44.11 ± 1.54	44.34 ± 1.57	0.434[1]
Corneal astigmatism (D)	1.01 ± 0.75	0.78 ± 0.49	0.192[2]
CCT[a] (μm)	557.75 ± 34.72	558.08 ± 30.10	0.958[1]
IOP-GAT (mmHg)	17.73 ± 2.86	16.77 ± 2.66	0.076[1]
IOPg (mmHg)	15.60 ± 3.19	15.24 ± 3.30	0.576[1]
IOPcc (mmHg)	16.28 ± 2.29	16.07 ± 3.29	0.733[1]
CH (mmHg)	10.20 ± 1.45	10.08 ± 1.22	0.637[1]
CRF (mmHg)	10.26 ± 1.49	10.05 ± 1.32	0.439[1]
WS	8.11 ± 1.21	8.30 ± 1.08	0.368[2]

Data were derived from [1]independent samples t-test, [2]Mann–Whitney U-test, and [3]chi-squared test. Continuous variables are reported as mean ± standard deviation. * $p < 0.05$ represents statistical significance.[a]CCT measured by using Pentacam at corneal vertex. CCT, central corneal thickness; CH, corneal hysteresis; CRF, corneal resistance factor; GAT, Goldmann applanation tonometry; IOP, intraocular pressure; Km, mean keratometry; mm, millimeters; n/a, not applicable; WS, waveform score; y, years; μm, micrometer.

it significantly increases on average 0.09 mmHg for each increase of 1 mmHg of IOPcc (Table 4).

4. Discussion

The authors present a cross-sectional study where they explored the corneal structural and biomechanical differences between subjects with and without type 2 DM. Our results revealed that IOPcc and CCT were the main parameters associated with corneal biomechanical properties, whereas DM type 2 was not a significant influencing factor. All results were confirmed on our multivariate assessments, adjusting for relevant confounders.

Since the publication of the first study, by Goldich et al. [11] in 2009, several other studies have addressed the effect of hyperglycemia on the corneal biomechanical properties of diabetic patients (Table 5). This subject has special clinical relevance for the growing incidence of diabetes worldwide and also the relevance that the cornea has in the measurement of IOP.

Previous work of Sady and colleagues [9] showed that hyperglycemia causes an increase in advanced Maillard products and oxidative stress resulting in increased collagen crosslinking. Moreover, they found that there was a decrease in the solubility of collagen by pepsin which could explain a reduced turnover of collagen and a consequent increase in corneal thickness and stiffness. However, it is important to note that CH or CRF does not reflect stiffness of corneal tissue [5]. With aging, there is also an accumulation of glycation end products and crosslinking of collagen molecules with increasing stiffness [28]; however, the reduction in the amount of proteoglycans and glycosaminoglycans of the extracellular matrix leads to a reduction in viscoelasticity and CH [18, 29]. On the other hand, in patients with diabetes, the washout of proteoglycans and glycosaminoglycans is reduced because they are more strongly connected and this is believed to increase the viscoelasticity and CH [14].

As pointed out previously in the introduction, the results regarding the influence of diabetes mellitus on the corneal biomechanics have not been consistent throughout the studies. One of the main reasons for this might be the high heterogeneity in subject characteristics across studies, in particular, type of DM and severity of DR. For example, Kotecha et al. [10] divided adult diabetic patients according to the type of DM, while other studies did not specify [11] or mix [12, 14, 16] type 1 and 2 patients without accounting for the important differences between them, such as DM duration. In the study by Kotecha and colleagues, the type 1 DM group was found to have significantly greater CH and CRF when compared to DM type 2 and non-DM subjects, with no statistical difference between the last two groups [10]. It is noteworthy that type 1 DM adult subjects had longer duration of DM in comparison with type 2 DM patients. In turn, two studies [26, 27] investigating corneal biomechanics in children with type 1 DM of short-term duration (<10 years) did not find any difference compared to controls (Table 5). In our study, only type 2 DM patients

Table 3: Subgroup analysis of diabetic patients.

		Age (y)	Female (n)	DM duration (y)	HbA1c (%)	DR presence (n)	CCT[a] (μm)	IOPcc (mmHg)	CH (mmHg)	CRF (mmHg)
Duration of diabetes (y)	<10 ($n = 30$)	73.30 ± 5.51	18 (60%)	4.90 ± 2.37	6.64 ± 0.75	4 (13%)	556.73 ± 38.15	16.41 ± 3.23	9.92 ± 1.29	9.96 ± 1.54
	≥10 ($n = 30$)	71.47 ± 5.75	20 (67%)	17.07 ± 7.00*	7.41 ± 1.32*	14 (47%)	558.77 ± 31.54	16.16 ± 3.39	10.49 ± 1.57	10.57 ± 1.38
HbA1c levels (%)	<7.0 ($n = 31$)	72.90 ± 5.21	18 (58%)	7.58 ± 5.75	6.20 ± 0.47	5 (16%)	553.74 ± 39.15	16.16 ± 3.50	10.02 ± 1.57	9.99 ± 1.49
	≥7.0 ($n = 29$)	71.83 ± 6.15	20 (69%)	14.62 ± 8.60*	7.91 ± 0.95*	13 (45%)	562.03 ± 29.33	16.42 ± 3.10	10.40 ± 1.32	10.56 ± 1.46
DR stage	No ($n = 42$)	72.95 ± 5.66	27 (64%)	8.38 ± 6.09	6.85 ± 1.05	—	555.69 ± 36.16	16.76 ± 3.35	10.07 ± 1.48	10.26 ± 1.62
	Yes ($n = 18$)	71.06 ± 5.58	11 (61%)	17.06 ± 8.87*	7.42 ± 1.26*		562.56 ± 31.53	15.17 ± 2.92	10.52 ± 1.37	10.27 ± 1.15

Continuous variables are reported as mean ± standard deviation. * $p < 0.05$ represents statistical significance. [a]CCT measured by using Pentacam at corneal vertex. CCT, central corneal thickness; K, keratometry; mm, millimeters; n/a, not applicable; WS, waveform score; y, years; μm, micrometer.

Table 4: Multivariate regression analysis of the relative effects of clinical and ocular characteristics on corneal biomechanical parameters: corneal hysteresis (CH) and corneal resistance factor (CRF).

Parameter	CH		CRF	
	B (95% CI)	p	B (95% CI)	p
Age (y)	−0.001 (−0.04 to +0.03)	0.965	−0.003 (−0.04 to +0.04)	0.879
Gender (male)	−0.24 (−0.64 to +0.17)	0.250	−0.303 (−0.80 to +0.19)	0.231
CCT (μm)	+0.02 (+0.01 to +0.02)	<0.001*	+0.02 (+0.01 to +0.03)	<0.001*
IOPcc (mmHg)	−0.21 (−0.27 to −0.15)	<0.001*	+0.09 (+0.02 to +0.16)	0.014*
DM duration				
Non-DM	—	—	—	—
DM2 < 10 y	−0.06 (−0.53 to +0.41)	0.792	−0.08 (−0.66 to +0.50)	0.792
DM2 ≥ 10 y	+0.411 (−0.05 to +0.87)	0.080	+0.48 (−0.08 to +1.05)	0.093

Data were derived from generalized linear models. * $p < 0.05$ represents statistical significance. CH, corneal hysteresis; CI, confidence interval; CRF, corneal resistance factor; D, diopters; DM, diabetes mellitus; mm, millimeters; y, years; μm, micrometer. The remaining variables (HbA1c, smoking history, BMI, AL, Km, and IOP-GAT) did not influence the model and were excluded.

were included but the differences from controls did not reach statistical significance, as in Kotecha et al.'s study.

In a recent population-based epidemiologic study, Schweitzer et al. [18] found that, in analyses adjusted for age, sex, and IOP, DM was associated with higher CH and CRF values; however, the effect was no longer significant after multivariate adjustment. According to the authors, these findings could be explained by a relatively small sample size of diabetic patients or a confounding effect of plasma LDL cholesterol. In our study, there were also no statistically significant differences between groups which might also reflect the relatively small size of the sample.

The large numbers of studies addressing corneal biomechanical behavior have faced its complexity and highlighted the importance of IOP as a major confounding variable in the assessment of corneal biomechanics using an air-puff stimulus [5]. In fact, our multivariate regression analysis confirmed the IOPcc and the CCT as the main parameters associated with corneal biomechanics properties. This is in line with previous studies that found CH to be positively associated with CCT and negatively associated with IOPcc, whereas CRF positively correlated with CCT and IOPcc [1, 21]. It is also important to recognize that the diabetes itself may also affect IOP and CCT [30]; therefore, we believe that including these covariates in the statistical models increased the confidence of our results and provided more robust conclusions. Importantly, none of the previous works who reported lower CH in diabetics adjusted CH or CRF for IOP, CCT, or age [16, 17, 19].

Scheler et al. [14] was the first to report that patients with poor glycemic control (HbA1c > 7%) had greater values of CH and CRF, controlling for IOP and CCT, compared to controlled DM (HbA1c <7%) and non-DM patients. Similarly, Yazgan et al. [15] reported the same results. Unfortunately, none of the studies provided information on disease duration of each DM group. In our study, longer DM duration was associated with greater HbA1c levels and presence of retinopathy, as expected; nevertheless, the sample included a low number of patients with prolonged DM duration (e.g., > 20 years) and advanced DR which might have influenced the results.

Our analysis failed to demonstrate a significant relationship between CH or CRF and age [29] as described in the literature; however, the CH and CRF values were smaller than other populations with younger samples (Table 5). The lack of a correlation may stem from the cross-sectional nature of our study and, also, the elderly population with short age-range included.

Table 5: Review of literature comparing corneal biomechanics obtained by ORA in patients with and without diabetes mellitus.

Study (year)	Nr of patients (eyes)		Glaucoma	Age (y)	Female (*n*)	DM diagn. duration	DR stage	HbA1c (%)	CCT (μm), US	IOPcc (mmHg)	CH (mmHg)	CRF (mmHg)
Goldich et al. [11] (2008)	Cont.	40 (40)	No	64 ± 9	19 (48%)	Interview Duration n.r.	n.r.	n.r.	530.3 ± 35.9	17.7 ± 4.9	9.3 ± 1.4	9.6 ± 1.6
	DM	40 (40)		61 ± 12	17 (43%)				548.7 ± 33.0*	16.6 ± 4.4	10.7 ± 1.6*	10.9 ± 1.7*
Hager et al. [12][a] (2009)	Cont.	195 (385)	26%	65 ± 16	206 (58%)[f]	Interview	Most patientsDR	n.r.	542.0 ± 40.0[d]	n.r.	10.4 ± 1.9	n.r.
	DM	50 (99)	20%	70 ± 11	42 (42%)[f]	13 ± 9 y			554.0 ± 50.0[d]		10.7 ± 1.7*	
Sahin et al. [16][b] (2009)	Cont.	61 (120)	No	53 ± 10	33 (54%)	Interview	n.r.	n.r.	535.5 ± 39.2	15.8 ± 3.2	10.4 ± 1.7	10.4 ± 2.0
	DM	43 (81)		55 ± 12	26 (60%)	14 ± 6 y		7.3 ± 1.5	550.1 ± 40.8*	18.8 ± 4.7*	9.5 ± 1.8*	10.3 ± 1.8
Kotecha et al. [10] (2010)	Cont.	123 (123)	No	54 ± 16	n.r.	Interview	n.r.	n.r.	550.1 ± 32.8	15.9 ± 2.7[e]	10.8 ± 1.7	10.6 ± 1.6
	DM 1	13 (13)		42 ± 11	18 (30%)	24 ± 15 y		7.3 ± 0.6	551.1 ± 27.2	15.4 ± 2.5[e]	12.4 ± 1.7*	12.5 ± 2.0*
	DM 2	48 (48)		62 ± 11		12 ± 10 y		7.2 ± 1.4	550.0 ± 40.9	16.2 ± 2.5[e]	10.9 ± 1.9	11.5 ± 2.1
Castro et al. [13] (2010)	Cont.	25 (40)	Yes	66 ± 15	16 (64%)	Interview Duration n.r.	No DR	n.r.	546.6 ± 37.3	n.r.	7.8 ± 1.7	n.r.
	DM 2	19 (34)		67 ± 9	14 (74%)				531.7 ± 31.3		9.1 ± 1.9*	
Scheler et al. [14][c] (2012)	Cont.	35 (35)	No	61 ± n.r	24 (69%)	Guidelines	n.r.	5.4 ± 0.5	n.r.	n.r.	10.7 ± 1.8	10.6 ± 2.2
	DM < 7%	14 (14)		66 ± n.r	14 (45%)	Duration n.r		6.0 ± 0.8			n.r.	n.r.
	DM ≥ 7%	17 (17)						8.6 ± 2.4			11.2 ± 2.1	12.2 ± 2.1*
Celik et al. [15] (2014)	Cont.	74 (74)	No	58 ± 10	50 (68%)	Guidelines	All DR stages	5.2 ± 0.6	551.4 ± 3.1	16.6 ± 0.4	9.0 ± 0.2*	9.0 ± 0.2*
	DM 2 < 7%	74 (74)		58 ± 10	40 (54%)	Duration n.r.		6.3 ± 0.3	543.0 ± 3.2	17.1 ± 0.4	9.8 ± 0.2*	10.1 ± 0.2*
	DM 2 ≥ 7%	82 (82)		58 ± 9	56 (68%)			9.9 ± 1.5	566.4 ± 3.0*	18.2 ± 0.3*	10.9 ± 0.2*	11.9 ± 0.2*
Pérez-Rico et al. [17] (2015)	Cont.	41 (41)	No	61 ± 9	31 (76%)	Guidelines	n.r.	5.2 ± 0.6	516.1 ± 34.0	14.6 ± 3.7	11.4 ± 1.7	10.5 ± 1.8
	DM 2 < 7%	40 (40)		62 ± 10	23 (58%)	12 ± 9 y		6.3 ± 0.3	561.3 ± 34.7	14.7 ± 2.7	10.9 ± 1.4	11.2 ± 2.0
	DM 2 ≥ 7%	54 (54)		60 ± 13	32 (59%)	15 ± 11 y		9.9 ± 1.5	565.2 ± 38.6	18.4 ± 3.8*	10.2 ± 1.8*	11.1 ± 2.0
Schweitzer et al. [18] (2016)	Cont.	695 (695)	No	>74[g,h]	-[g]	Guidelines Duration n.r	n.r.	n.r.	548.9 ± n.r.	-[g]	9.3 ± *n*.r.	9.6 ± *n*.r.
	DM 2	137 (137)							558.2 ± n.r.		9.8 ± *n*.r.*	10.4 ± *n*.r.*
Bekmez and Kocaturk [19] (2018)	Cont.	50 (50)	No	62 ± 12	26 (52%)	—	—	—	—	16.0 ± 3.1	10.5 ± 1.7	10.5 ± 1.7
	DM 2	50 (50)		63 ± 9	25 (50%)					17.8 ± 3.6*	9.9 ± 1.5	10.4 ± 1.6

Table 5: Continued.

Study (year)	Nr of patients (eyes)		Glaucoma	Age (y)	Female (*n*)	DM diagn. duration	DR stage	HbA1c (%)	CCT (μm), US	IOPcc (mmHg)	CH (mmHg)	CRF (mmHg)
Kara et al. [26] (2013)	Cont.	50 (50)	No	15 ± 2	31 (62%)	Guidelines	No DR	n.r.	559.0 ± 22.0	15.1 ± 2.7	12.5 ± 1.5	11.9 ± 1.5
	DM 1	46 (46)		14 ± 2	26 (57%)	6 ± 3 y		10.4 ± 2.4	555.0 ± 26.0	15.5 ± 3.4	12.3 ± 1.3	12.4 ± 1.7
Nalcacioglu-Yuksekkaya et al. [27] (2014)	Cont.	74 (74)	No	13 ± 3	48 (65%)	Guidelines	No DR	n.r.	n.r.	15.3 ± 3.4	10.7 ± 1.7	10.5 ± 1.6
	DM 1	68 (68)		13 ± 3	34 (50%)	5 ± 3 y		8.3 ± 2.0		15.8 ± 3.0	10.8 ± 1.5	10.9 ± 1.9

*Statistical significant difference at $p < 0.05$. CCT, central corneal thickness; diagn., diagnosis; DR, diabetic retinopathy; SD, standard deviation; US, ultrasound paquimetry; y, years; mmHg, millimeters of mercury. [a]Retrospective study. [b]The study included 22 type 1 DM and 21 type 2 DM patients. [c]The study included 3 type 1 DM and 28 type 2 DM patients. [d]CCT was measured with Orbscan; [e]IOPcc was not reported, and the values of IOP were measured with a dynamic contour tonometer. [f]Number of eyes included in the study. [g]CCT, CH, and CRF values were adjusted for age, sex, and IOP; [h]Mean age was comparable between groups; [i]Values are reported as mean ± standard error.

Finally, as DM diagnosis, especially type 2, depends on various factors such as knowledge of risk factors and access to the health system, the real time from onset to diagnosis might be unknown in some patients. This is particularly relevant, as corneal changes might correlate with duration of DM and glycemic control. In our study, all patients regularly attended primary care physicians which might have reduced the selection bias.

In conclusion, the CCT and IOPcc were found to be the main variables that affect corneal biomechanical properties both in diabetic and controls, whereas type 2 DM had no significant effect. The ORA has proven to be an easy-to-use tool that can be incorporated into daily clinical practice to provide important data in patient assessment. Further prospective studies with larger samples and control of confounding factors are required to better understand the relationship between long-term poor glycemic control and corneal biomechanics changes.

Data Availability

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

Disclosure

Part of this paper was presented at the Congress of the European Society of Ophthalmology (SEO), Barcelona, Spain, 10–13 June 2017.

Conflicts of Interest

Manuel S. Falcão has participated in advisory boards for Bayer and has received travel grants from Novartis, Alimera, and Allergan. Angela Carneiro has participated in advisory boards for Alcon, Bayer, Novartis, Alimera, and Allergan. The other authors declare no conflicts of interest regarding the publication of this paper.

Authors' Contributions

JNB, MF, VR, AC, and FFR were involved in study concept and design. JEL, DR, and RM participated in data collection. JNB, JEL, MF, AC, VR, and FFR carried out analysis and interpretation of data. JNB, JEL, DR, and RM participated in drafting of the manuscript. MF, VR, AC, and FFR were responsible for critical revision of the manuscript. JNB and MF carried out statistical expertise.

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Research Article

Influence of Type 1 Diabetes Mellitus on the Ocular Biometry of Chinese Children

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Purpose. To compare ocular biometry between children with type 1 diabetes mellitus (T1DM) and healthy children in China and to determine the correlation of ocular biometry with the glycosylated hemoglobin (HbA1c) level and diabetes duration. *Methods*. A case-control study was conducted at Children's Hospital of Fudan University between T1DM children and healthy children. The participants were evaluated for central corneal thickness (CCT), anterior chamber depth (ACD), lens thickness (LT), K1 and K2 keratometry, and axial length (AL); also cycloplegic refraction was performed, and spherical equivalent (SE) was acquired. HbA1c levels of the T1DM cases were obtained. *Results*. Fifty-four eyes of 54 children with T1DM and 53 eyes of 53 healthy children were included. The mean age of T1DM group and control group was 10.59 ± 3.40 years and 9.55 ± 1.89 years, respectively, and the differences between age and gender were not significant ($p = 0.052$, $p = 0.700$). The mean LT in T1DM group (3.49 ± 0.18 mm) was thicker than that in the control group (3.40 ± 0.16 mm) ($p = 0.018$), the mean ACD in T1DM group (3.52 ± 0.26 mm) was shallower than that in the control group (3.72 ± 0.26 mm) ($p < 0.001$), and there were no significant differences of CCT, K1, K2, AL, and SE ($p = 0.088$, $p = 0.672$, $p = 0.821$, $p = 0.094$, and $p = 0.306$, respectively). There was no significant correlation between HbA1c or diabetes duration and ocular biometry. *Conclusions*. Thicker LT and shallower ACD occurred in T1DM children rather than age-matched and sex-matched healthy children, but the overall refraction was not affected. HbA1c or diabetes duration was not correlated with ocular biometry in T1DM children.

1. Introduction

Diabetes mellitus (DM) may lead to multisystem complications, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Eye diseases such as cataract, glaucoma, keratopathy, refractive changes, oculomotor nerve paralysis, or diabetic retinopathy (DR) are associated with DM. Although DR is the most noteworthy complication as its threatening outcome is premature blindness, it is rare in children regardless of duration and control of DM. In DM patients, the optical quality might be deteriorated as the tear film, cornea, crystalline lens, and the vitreous are susceptible to hyperglycemia [2, 3]. These changes could be asymptomatic for type 1 diabetes mellitus (T1DM) children, especially when children are young. Axial length, corneal radius of curvature, and lens thickness were the most important determinants of refraction [4]. There is evidence that lens is susceptible in T1DM adults [5, 6]. For growing children, would deteriorations for refractive components affect the refractive development? A previous study indicates that the proportion of myopia is higher in T1DM children aged less than 10 years, but not in older age, and poor glycemic control is not related to higher myopia risk [7]. How does myopia be accelerated in T1DM children? Which components are suffering and leading to this risk, the cornea, the lens, or axial length? Would there be a correlation of the DM

condition and the refractive components? Our study aimed to reveal how does T1DM affect children's refractive status.

2. Methods

This was a hospital-based case-control study approved by the ethics committee of both Children's Hospital of Fudan University in Shanghai (approval number: No. 01 (2018)) and Shanghai General Hospital (approval number: 2016KY005). This study conformed to the guidelines proposed in the Helsinki Convention. It was a part of the Shanghai Children and Adolescent DM Eye study (SCADE).

Fifty-four eyes of 54 patients with T1DM and 53 eyes of 53 healthy subjects were included in this study. Eligible participants with T1DM were screened from the electronic medical record system of Children's Hospital of Fudan University where they had previously been diagnosed by the endocrine department according to the criteria of the American Diabetes Association [1] and were contacted by telephone to encourage participation. Healthy children were chosen from the ones who had presented to our hospital for routine vision examination. Written informed consent was obtained from each participant at the examination site.

The patients in the T1DM group were under 18 years old and were diagnosed with T1DM at least 1 year before examination. We excluded those with other metabolic disorders (i.e., Prader–Willi syndrome) and contact lens wearers (i.e., orthokeratology lens). Eyes with history of ocular trauma and diseases (i.e., corneal pathology, cataract, glaucoma, optic nerve atrophy, retinopathy, and strabismus) were also excluded. Children in the control group were under 18 years old with normal ocular findings and without systemic problems, and contact lens wearers were also excluded.

Before measurements, a questionnaire was conducted in written form. History of general and eye disease, DM type and complications, onset age and duration, height and weight, blood glucose control method, recent blood glucose level, etc., was reflected in the inquiry.

Examinations were performed by 3 experienced ophthalmologists and 2 optometrists. All of the participants underwent a comprehensive eye examination all in the same day. Before cycloplegia, eye movement and eyelid were checked up; anterior segment was examined using slit lamp biomicroscope; visual acuity (VA) was measured in both eyes using a retro-illuminated logarithmic visual acuity chart; refractive error and K1 and K2 keratometry were measured by an autorefractor (ARK-1; Nidek, Tokyo, Japan); intraocular pressure and central corneal thickness (CCT) were measured by a pneumotonometer (NT-530P; Nidek, Tokyo, Japan); anterior chamber depth (ACD), lens thickness (LT), and axial length (AL) were acquired by IOL (intraocular lens) Master (700; Carl Zeiss Meditec, Dublin, CA); and nonmydriatic fundus photograph was taken by a digital camera (AFC-210; Nidek, Tokyo, Japan). Then, pupil was dilated by 1% cyclopentolate. After that, subjective refraction was performed, and the refraction data was converted to spherical equivalent (SE; SE = sphere power + 1/2 cylinder power). Macular scan was taken by optical coherence technology (OCT) (RS-3000; Nidek, Tokyo, Japan), and fundus blood flow was observed by swept-source OCT angiography (Triton; Topcon, Tokyo, Japan). For all the T1DM patients, fasting venous blood was obtained for determination of serum glycosylated hemoglobin (HbA1c) level.

Statistical analysis of the data was performed with SPSS version 21.0. Mean values and standard deviation (SD) were used for descriptive analyses. Because of the significant correlation between the right and left eyes, only the right eyes were used for the statistical analysis. Kolmogorov–Smirnov test revealed no significant deviation from a normal distribution for all of the test parameters. Independent t-test was used to compare age and ocular parameters between the study group and control group. Chi-square test to compare gender, Pearson correlation, and multiple linear regression model were used to determine the correlation between anterior ocular segment biometry and HbA1c level or DM duration. Statistical significance was set as $p < 0.05$.

3. Results

The mean age of the T1DM group and control group was 10.59 ± 3.40 years (range 5–17 years) and 9.55 ± 1.89 years (range 5–13 years), respectively; there were 25 male and 29 female patients in the T1DM group and 27 male and 26 female healthy children in the control group, the differences between age and gender of the 2 groups were not significant ($p = 0.052$, $p = 0.700$). The mean duration of DM was 4.19 ± 2.69 years (range 1–12 years), and the mean HbA1c level at the time of the study was $7.71\% \pm 2.23\%$ (range 4.6%–14.1%) in the T1DM patients. The mean value of LT in the T1DM group and control group was 3.49 ± 0.18 mm and 3.40 ± 0.16 mm, respectively; the LT was significantly thicker in the T1DM group ($p = 0.018$, α 0.05, power 0.825). There was also significant difference of ACD ($p < 0.001$, α 0.05, power 0.977) between 2 groups, the ACD of control group was 3.72 ± 0.26 mm, which was much deeper than 3.52 ± 0.26 mm of the T1DM group. There were no significant differences of CCT, K1, K2, AL, and SE in 2 groups ($p = 0.088$, $p = 0.672$, $p = 0.821$, $p = 0.094$, and $p = 0.306$, respectively) (Table 1). Diabetic retinopathy was not seen in any of the patients.

Table 2 shows the correlations among each ocular parameter in 2 groups and the correlations between HbA1c or duration of DM and ocular parameters in T1DM group. Since age was correlated with ACD, LT, AL, SE, HbA1c, and DM duration, age was adjusted besides CCT, K1, and K2 with the multiple linear regression model when there were significant correlations with Pearson correlation. It could be seen that neither HbA1c nor DM duration was correlated with ocular biometry in T1DM group, CCT was not correlated with any of the other parameters in both group, ACD had positive effect on AL in both groups, LT had negative effect on ACD in DM group, and AL had negative effect on SE in both groups.

4. Discussion

Overt but asymptomatic changes occur in LT and ACD of T1DM children preceding cataract, glaucoma, DR, and even

Table 1: Ocular parameters of T1DM patients and healthy controls.

	DM group (mean ± SD)	Control group (mean ± SD)	t value	p value
Age, years	10.59 ± 3.40	9.55 ± 1.89	1.963	0.052[a]
M/F, n	25/29	27/26	—	0.700[b]
CCT, μm	560.29 ± 29.29	571.02 ± 31.61	−1.722	0.088[a]
ACD, mm	3.52 ± 0.26	3.72 ± 0.26	−4.104	0.000[a]
LT, mm	3.49 ± 0.18	3.40 ± 0.16	2.422	0.018[a]
AL, mm	23.86 ± 1.36	24.28 ± 1.20	−1.691	0.094[a]
K1, D	42.11 ± 1.69	41.97 ± 1.48	0.425	0.672[a]
K2, D	43.14 ± 1.76	43.22 ± 1.63	−0.227	0.821[a]
SE, D	−1.13 ± 2.45	−1.59 ± 1.96	1.030	0.306[a]

CCT: central corneal thickness; ACD: anterior chamber depth; LT: lens thickness; AL: axial length; K1: flat meridian; K2: steep meridian; SE: spherical equivalent; D: diopters; DM: diabetes mellitus; SD: standard deviation. [a]Independent t-test. [b]Chi-square test. p value < 0.05 significant.

Table 2: Correlation coefficients among ocular biometry, HbA1c level, and DM duration.

	ACD (mm)	LT (mm)	AL (mm)	K1 (D)	K2 (D)	SE (D)	HbA1c (%)	DM duration (years)
CCT, μm								
DM	−0.073	0.277	−0.084	0.043	0.039	0.080	−0.117	0.154
Control	0.033	−0.261	0.168	−0.089	−0.011	−0.031	—	—
ACD								
DM		−0.339**[a]	0.387**[a]	−0.059	−0.064	−0.206[a]	0.112	0.004
Control		−0.124[a]	0.308**[a]	0.128	0.091	−0.214[a]	—	—
LT								
DM			0.011[a]	−0.108	−0.115	−0.045[a]	−0.114	−0.097
Control			−0.149[a]	−0.091	−0.099	0.253[a]	—	—
AL								
DM				−0.364**	−0.263	−0.863**[a]	0.010	0.237
Control				−0.375*	−0.307	−0.661**[a]	—	—
K1								
DM					0.945**	0.304*	0.223	0.010
Control					0.939**	−0.192	—	—
K2								
DM						0.104	0.164	0.050
Control						−0.227	—	—
SE							0.089	−0.064[a]
HbA1c							—	0.234[a]

CCT: central corneal thickness; ACD: anterior chamber depth; LT: lens thickness; AL: axial length; K1: flat meridian; K2: steep meridian; SE: spherical equivalent; D: diopters; DM: diabetes mellitus; HbA1c: hemoglobin A1c. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). [a]Beta values by multiple linear regression models, other data are r values by Pearson correlation.

SE change. Although significant myopia difference could not be read from the SE, underlying mechanism of T1DM is occultly ruining refractive components from our study. Whether these are signs of other complications is still unknown. DR screening examinations for T1DM children are suggested to begin at age 15 years or at 5 years after the diagnosis of DM [3]; however, earlier attention should be paid to refraction. SCADE is a study aiming to investigate the ocular disorders of DM children since January 2018; we will follow up the changing trends in ocular biometry, and the present study provided groundwork and also an inspiration to our research in the future.

The present study showed that the LT was significantly larger accompanied by the ACD decrease than that of the healthy children, which agree with the findings of Uzel et al. [8]. A previous study of internal structure of lens performed with corrected Scheimpflug imaging by Wiemer et al. [9] found that the lens was consisted of three cortical zones and the nucleus; in T1DM patients, all four layers rather than one typical layer of the lens were significantly thicker compared with those of the healthy control subjects, which supports the hypothesis that the thickening of the lens is the result of cellular or extracellular overhydration rather than insulin-induced mitogenesis of the epithelial cells. However, in contrast to T1DM, the lens of type 2 diabetes mellitus (T2DM) patients showed no difference compared with control lens for all layers. This suggests that T1DM and T2DM have different underlying pathophysiologic mechanisms. Does the lens grow larger on account of swelling in T1DM patients? A study used MRI scan by Adnan et al. [10] found the differences in lens shapes between the T1DM and control groups, the diabetes had more rounded shapes with smaller equatorial diameters and greater axial thicknesses; meanwhile, the amplitude of accommodation was smaller, which means the zonules are on greater tension and the ciliary muscles are less contractive on the diabetic eyes. Wiemer et al. [6] also found a more convex lens in T1DM patients that the lens were thicker and both the anterior and posterior radii were smaller. So, the lens becomes rounder rather than larger in T1DM patients. The aforementioned

authors also reported a ACD decrease accompanied by the LT grew [6, 8, 10].

On one hand, T1DM had a profound effect on lens; however, CCT, K1, and K2 remained unchanged in T1DM children in the present study. With regard to corneal stroma is a highly hydrophilic structure, it is crucial for epithelium and endothelium to play the role in blocking the penetration of polarized substances from getting into cornea, and the endothelial pumping mechanisms is also vital to maintain corneal dehydration. The DM-caused epithelium/endothelium abnormalities include a decrease in the number of cells, polymorphism, polymegathism, and increase in the cellular coefficient of variation, which affect the barrier functions; hyperglycemia is known to inhibit Na/K ATPase-dependent transport of the endothelial cells. It is hypothesized that these changes will lead to corneal hydration and swelling [2, 11]. Some previous studies reported an increased CCT in DM patients than non-DM, regardless of retinopathy status. For example, Suraida et al. [12] found that there was significant mean difference of CCT between non-DM and DM with nonproliferative diabetic retinopathy (NPDR) or no DR in T2DM patients; the NPDR group showed the highest CCT of 529.26 μm, then 524.60 μm for the no DR group, and 493.12 μm for the non-DM group. It differed from the study by Uzel et al., who found patients with juvenile DM had similar CCT, K1, and K2 compared to age- and sex-matched healthy children, the mean CCT value was 542.95 μm and 541.38 μm, respectively [8]. To concur with that, Wiemer et al. [13] measured CCT from 102 patients with T1DM, 101 patients with T2DM, and 69 healthy subjects, and the mean CCT was 0.578 mm, 0.586 mm, and 0.578 mm respectively; no statistically significant difference was found between the 3 groups, whilst the anterior radius and overall corneal power did not differ significantly except for the posterior corneal radii between the 3 groups. Unlike the wide agreement in lens changes of DM patients, it is still controversial whether corneal thickness is affected in DM patients. The discrepancies among different reports could be different devices that are used in CCT measurement, and the HbA1c level may determine the agreement of each pachymetry devices according to Altay et al. [14]. The outcomes in our study were similar to Wiemer et al. and Uzel et al., the mean CCT, K1, and K2 showed no significant difference between 2 groups. Unlike the aforementioned studies, our study showed a marginal significant difference of CCT ($p = 0.088$) with mean value 571.02 μm of the control group a little higher than 560.29 μm of the T1DM group; the true reason for this is unknown, and further researches with larger sample size would be needed.

In present study, the AL and SE showed no significant difference between the T1DM group and the control group. Among growing Chinese children from 7 to 14 years, usually a 0.19 mm decrease appears in LT, and increased myopia was related to increases in AL and LT and to decreases in corneal radius of curvature [4]. Given comparatively stable corneal power and AL, a greater LT in T1DM children could be a thinkable risk for myopia. It was concluded by Duke-Elder in 1925 [15] that hyperglycaemia led to myopia, while lowering the blood glucose led to hyperopic. This was proved in the latter ex vivo bovine lens research by Mehta et al. that a trend towards myopia was observed with increasing hyperglycaemia and a hyperopic shift was observed as the glucose return to normal [16]. Nevertheless, there was also reported a myopic shift after a relative hypoglycaemia. Yarbağ et al. [17] found in newly diagnosed T2DM, the average refractive value was +2.50 diopters, and after four weeks' treatment, the average refractive value turned out to be +0.75 diopters as the plasma glucose level went down. So, the question arose whether a decrease in equivalent refractive index of the lens compensated for convex lens shape. Wiemer et al. calculated the equivalent refractive index of the lens in T1DM and T2DM and found a significant decrease in the equivalent refractive index of lenses compared with the control group in T1DM but not in T2DM and combined with more convex lens shape in T1DM and no lens shape change in T2DM, resulting in no lens power change in 2 types of DM [6]. In agreement with that, Adnan et al. revealed a significant decrease in the equivalent refractive index of lenses and no significant change in lens equivalent power in T1DM patients compared with non-DM controls [5]. Lens power could not be directly measured but could be calculated from the refractive indices of the aqueous, lens, vitreous, LT, and the radius of anterior and posterior lens surface [5, 6]. Apparently, LT is one of the determinant factors of lens power yet could not represent lens power. As shown in (Table 2), LT alone was not correlated with SE from our study. It could be deduced that for newly diagnosed DM, hyperglycaemia leads to myopia, this is a transient phenomenon caused by initial lens power increases; however, lens power decreases with the plasma glucose return, and there may be lags for lens power return as the myopic shift was observed after a few weeks' treatment by Yarbag. But the lens status differs for long-term DM with relatively stable plasma glucose. For T1DM, lens refractive index goes down whereas convexity increases; for T2DM, lens shape stays as usual with unchanged lens refractive index as the healthy control. To conclude, lens power increases in uncontrolled hyperglycaemia and the compensation theory exists in T1DM patients. The T1DM children in our study were with at least one year of DM duration, LT increased while SE remained the same compared with healthy controls, and these could be the compensation from lens refractive index.

It was noteworthy that despite LT and ACD significantly changed in T1DM children, no relation was found between the blood HbA1c level or DM duration and LT, ACD, and the other unchanged parameters (AL, SE, K1, K2, and CCT). Our study was in agreement with Uzel et al.'s [8], who found no relationship between LT and the HbA1c level in T1DM children. Several other studies have investigated the effect of DM duration or HbA1c level on ocular parameters. Adnan et al. [5] assessed it in multiple regression fits, and the duration of diabetes contributes to ACD, LT, and lens equivalent refractive index but not contributes to SE, CCT, and lens equivalent power in T1DM adults. In line with Adnan, Wiemer et al. revealed that, in T1DM adults, the duration of DM was found to have significant influences on the ACD, LT, lens refractive index, and lens anterior and posterior curvature, while no associations were found

between the duration of DM and the ocular refraction, CCT, and corneal radius; HbA1c was explored to have no significant influence on the various lens and corneal parameters [9, 13]. In the study of Chinese T2DM by Song et al. [18], blood levels of HbA1c were not related to AL, ACD, and corneal radius. It could be concluded that, in T1DM patients but not T2DM patients, lens parameters were sensitive and corneal parameters were apparently stable; for T1DM children, DM duration was too short to have profound effect on lens changes compared to adults; however, for adults, besides DM duration, aging could have an inevitable impact on long-term changes too, and HbA1c level had no effect on ocular parameters of DM patients.

Our study has limitations. This is a cross-sectional data analysis; it only represents ocular status at examining time, because of the sensitivity of lens parameters of T1DM children, fluctuation is possible, and serial ocular biometry measurements are useful in further study. For growing children, ocular biometry is changing along with growth; repeated measurements after a period of time could be helpful to reveal the changing trends besides growth. Larger-scale and multicenter study may be needed to better elucidate how these changes affect the refractive development, and consensus should be made that when shall we start to monitor the refractive development of T1DM children if necessary.

5. Conclusions

In T1DM children, we found that LT became larger accompanied by ACD decrease, while the other ocular biometry was apparently unaffected; however, the overall refractive error remained unchanged. We deemed this as a compensation from the lens refractive index. DM duration and HbA1c level did not affect ocular biometry.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Disclosure

The authors alone are responsible for the content and writing of the paper.

Conflicts of Interest

The authors report no conflicts of interest.

Authors' Contributions

Ying Xiao and Tao Li contributed equally to this work.

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Research Article

The Effect of *Buddleja officinalis* Maxim Eye Drops on Morphology and Apoptosis in Lacrimal Gland of Experimental Dry Eye Rabbit Model

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The purpose of this study was to investigate the effects of *Buddleja officinalis* Maxim eye drops on morphology and apoptosis in lacrimal glands of the experimental dry eye rabbit model. A total of thirty-six male rabbits were divided into six study groups, consisting of the control group and the dry eye rabbit model group (without any treatment), the dry eye rabbit model group treated with testosterone, and the dry eye rabbit model group treated with different concentrations of *Buddleja officinalis* Maxim eye drops (1.0 mg/ml, 1.5 mg/ml and 3.0 mg/ml). The lacrimal glands were evaluated by hematoxylin-eosin staining and immunohistochemistry. *Buddleja officinalis* Maxim eye drops can improve the morphological structure of the lacrimal gland in the dry eye model of castrated rabbits. The average optical density values of PI3K, Akt, and caspase-9 protein in the lacrimal gland tissue of the 3 mg/ml *Buddleja officinalis* Maxim eye drops group were significantly different from those in the model group ($P < 0.01$) and similar to the testosterone control group and the control group ($P > 0.05$). *Buddleja officinalis* Maxim eye drops can improve the morphological structure of the lacrimal gland in the dry eye model of castrated rabbits.

1. Introduction

Dry eye disease (DED) is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles [1]. At present, it has become the most common eye disease except refractive error. Some studies have confirmed that decreased androgen levels are an important factor in the dry eye [2].

The basic treatment principle of the dry eye is to improve the symptoms of eye discomfort and protect the patient's visual function, by supplementing or restoring the normal components of tears, restoring the normal anatomy of the surface of the eye, inhibiting the inflammation of the surface of the eye, and finally recovering the ocular surface and the tear film, so as to reach the normal anatomy and physiological function [3]. Currently, we used treatment methods including physical therapy, intense pulsed light (IPL), moisture chamber glasses, artificial tears, lipid replacement therapy, anti-inflammatory therapy, and lacrimal duct

embolism [4], but the efficacy is not satisfactory, and even caused many side effects. Therefore, Chinese medicine with abundant natural medicine resources has broad prospects in the field of dry eye treatment and has great potential for discovering innovative drugs.

Buddleja officinalis Maxim is a kind plant of the genus *Buddleia*, and we can extract 8 flavonoids from its flower buds, which is a type of phytohormone. Flavonoids can bind to the androgen receptor to act as an androgenic activity because it has the same chemical structure as androgen-heterocyclic polyphenols and to maintain the normal level of androgen in the body to treat some diseases caused by decreased androgen levels [5]. Our previous experimental study confirmed that *Buddleja officinalis* Maxim granules can downregulate the expression of apoptotic factors Bax, Fas, and FasL in ovariectomized rabbit lacrimal gland cells and upregulate the expression of Bcl-2, thereby inhibiting the apoptosis of lacrimal gland cells and maintaining the basis amount of secretion of the lacrimal gland, but its effect is weaker than androgen [6]. The related preparations of *Buddleja officinalis* Maxim granules and their medicinal herbs have a positive therapeutic effect on the dry eyes caused by decreased androgen levels. Therefore, we suspect that it may also have a certain effect on the treatment of the dry eye caused by decreased sex hormone levels.

This study mainly studied the effects of *Buddleja officinalis* Maxim eye drops on the morphology of the lacrimal gland and the apoptosis of lacrimal gland cells in the castrated rabbit dry eye model, and further explored the exact effect of *Buddleja officinalis* Maxim eye drops on the dry eye and its possible mechanism to seek new treatments of the dry eye.

2. Materials and Methods

2.1. Animals. There were six rabbit groups (totally thirty-six rabbit, $n = 6$ rabbit each), i.e., the control group (male rabbit without any treatment), the dry eye model rabbit group, dry eye rabbit group treated with testosterone, the dry eye rabbit model group treated with 1 mg/ml *Buddleja officinalis* Maxim eye drops, the dry eye rabbit model group treated with 1.5 mg/ml *Buddleja officinalis* Maxim eye drops, and the dry eye rabbit model group treated with 3 mg/ml *Buddleja officinalis* Maxim eye drops. The administration of *Buddleja officinalis* Maxim eye drops was performed for a month.

2.2. Dry Eye Model of Rabbit due to Castration. To perform castration, male New Zealand white rabbits were anesthetized using 25% urethane (4 ml/kg). Anesthesia was satisfactory and then fixed on the stent. Testicular area was a disinfected, aseptic surgical procedure. We squeezed one side of the testicle from the abdominal cavity into the scrotum and fixed it and then using a sterile blade to make an incision in the scrotum area, forcefully extruded the testicle and ligatured the spermatic vein and the vas deferens and then removed the testis and epididymis and applied a proper amount of penicillin powder to prevent infection, continuous suturing of scrotal skin tissue after local disinfection. The other side of the testis and epididymis removal method is the same [7, 8].

2.3. Drugs

2.3.1. Total Flavonoids and Total Phenylpropanoid Extracts of Buddleja officinalis Maxim. Total flavonoids and total phenylpropanoid extracts of *Buddleja officinalis* Maxim are prepared by the Department of Pharmacy, the Second Xiangya Hospital of Central South University. The specific extraction process is as follows: we used 60% ethanol to extract 100 kg *Buddleja officinalis* Maxim twice in a row. We added 10 times the volume of ethanol for the first time and 8 times the volume of ethanol for the second time, combined the ethanol extract, recovered the ethanol under reduced pressure to the taste of no alcohol, treated the concentrate with the ZTC clarifying agent, filtered it, filtered the HPD-100 macroporous resin column, and eluted with different concentrations of ethanol. The 60% ethanol eluate was dried in vacuum and pulverized to obtain 1.5 kg of dry paste.

2.3.2. Buddleja officinalis Maxim Eye Drops. *Buddleja officinalis* Maxim eye drops is prepared by the College of Pharmacy of Hunan University of Chinese Medicine, and the extracts of *Buddleja officinalis* Maxim is prepared according to the eye drop preparation process. We dissolved the total flavonoids and total phenylpropanoid extracts of *Buddleja officinalis* Maxim with injection water and prepared them by microporous filtration membrane filtration and sterilizing filtrate. According to the preliminary experimental results of our research group, we decided to select five concentrations (3 mg/10 ml, 5 mg/10 ml, 10 mg/10 ml, 15 mg/10 ml, and 30 mg/10 ml). The eye drops were prepared without any preservatives and stored in a 4–8°C refrigerator for about 1 week. We used the *Buddleja officinalis* Maxim eye drops 3 times a day: about eight o'clock, twelve o'clock, and sixteen o'clock.

2.3.3. Testosterone Propionate Injection. Testosterone propionate injection is produced by Tianjin King York Pharmaceutical Co., Ltd., of China (specification: 1 mL: 25 mg). At present, there is no testosterone eye drops applied to the clinic. In Group F, all experimental animal are injected once in every 3 days to reach and supplement the role of androgen, and 2 mg/kg testosterone propionate injection is injected into the muscles of rabbit thighs (in terms of human and rabbit body surface area [9]).

2.4. Schirmer's Test I. Schirmer's test I (SIT) values were measured and recorded in each experimental group before modeling, 8 wk after modeling, and 14 d and 28 d after medication. SIT, according to the instructions, the tear secretion test paper was placed at the junction of the outer and outer 1/3 of the conjunctival sac under the eye; gently close the eyes, remove the filter paper after 5 minutes, and measure the wet length of the filter paper, calculated in millimeters.

2.5. Diagnostic Criteria for Dry Eye in China. One of the subjective symptoms such as dryness, foreign body sensation, burning sensation, fatigue, and vision fluctuation, and

BUT ≤5s or Schirmer I test (no surface anesthesia) ≤5 mm/5 min can diagnose the dry eye [10].

2.6. Experimental Process. The experimental animals were fed adaptively in the laboratory for one week to exclude the animals in poor health. The other animals were randomly divided into 6 groups with 6 animals in each group. Except for the control group, the other 5 groups were made models (bilateral testiculectomy and epididymis excision). The test of tear secretion after two months of feeding proved that the animal model was successfully established, and the drug was given on the first day after the successful establishment of the animal model. The control group without any treatment (Group A), the dry eye model rabbit group without any treatment (Group B), the dry eye rabbit model group treated with 1 mg/ml *Buddleja officinalis* Maxim eye drops (Group C, three times a day), the dry eye rabbit model group treated with 1.5 mg/ml *Buddleja officinalis* Maxim eye drops (Group D, three times a day), the dry eye rabbit model group treated with 3 mg/ml *Buddleja officinalis* Maxim eye drops (Group E, three times a day), dry eye rabbit group treated with testosterone (Group F, once every three days) had continuous administration for one month. At the end of the experiment, the lacrimal gland of the animal was removed.

2.7. Removing the Lacrimal Gland. New Zealand white rabbits were anesthetized with 25% urethane (4 ml/kg) via ear vein. Anesthesia was satisfactory and then fixed on the stent to remove the lacrimal glands. The lacrimal gland tissue was fixed in formaldehyde fixative solution and sent to the immunohistochemical test. After the end of the experimental, the animals were sacrificed by air embolism.

2.8. Light Microscopic Observation of the Morphology of the Lacrimal Gland. The lacrimal gland was immediately placed in 4% paraformaldehyde, embedded in paraffin, and sectioned with hematoxylin-eosin staining. The lacrimal gland structure of each group of New Zealand white rabbits was observed under light microscope.

2.9. Immunohistochemistry Was Used to Detect the Expression of Apoptotic Factors PI3K, AKT, and Caspase-9 in Lacrimal Gland Cells of Castrated Rabbits. After the lacrimal gland specimen is dewaxed and hydrated, it is operated according to the instructions of the immunohistochemistry kit. The expression of caspase-9, Akt, and PI3K protein was observed under the optical microscope, and 12 slices were randomly selected from each group. Five 400-fold fields were randomly observed for each slice. All positive particles in the field of view were photographed and accurately selected. The average optical density value was obtained by Image-pro Plus 6.0 analysis software and used to quantitatively express the degree of immunocytochemical reaction. Judging criteria: the cytoplasm staining of positively labeled cells after immunohistochemistry was brown or dark brown. The image analysis system was used for image analysis and processing. The above detection is done through PV-9000 2-step plus Poly-HRP Anti-Mouse/Rabbit IgG Detection System.

2.10. Statistical Analysis. Experimental data analysis was performed using SPSS 25.0 system software. All data of the experimental results were expressed as mean ± standard deviation ($\overline{x} \pm \mathrm{SD}$). The multiple sets of comparisons satisfy the normal and the variance homogeneity, and the variance analysis is used. If the normality and homogeneity of variance are not satisfied, the rank sum test is used. $P < 0.01$ is considered to be statistically significant.

3. Results

3.1. Comparison of SIT Values before and after Modeling in Male Rabbits. Before the animal model was built, the SIT values of each group were normal, and there were no difference between the groups ($P > 0.05$). 8 weeks after modeling, the SIT values of 1.0 mg/ml, 1.5 mg/ml, and 3.0 mg/ml *Buddleja officinalis* Maxim eye drops and the testosterone group were significantly decreased, compared with the control group, and the difference was statistically significant ($P < 0.01$) and reached the diagnostic criteria for the dry eye. 14 days after treatment, the SIT values of the treatment groups increased slightly, compared with the model group, and there was no significant difference ($P > 0.05$). 28 days after treatment, the SIT values of the treatment groups increased significantly, of which 1.5 mg/ml *Buddleja officinalis* Maxim eye drops group was similar to the testosterone group ($P > 0.05$) (Table 1).

3.2. Light Microscopic Observation of the Morphology of Lacrimal Gland. After the end of the experiment, the lacrimal gland tissue was stained by HE and then observed under the light microscope.

3.2.1. Control Group. The connective tissue of the lacrimal gland divides the glandular tissue into small leaflets of different sizes. The lacrimal gland has a clear structure, and the acinar and lacrimal gland epithelium are uniform in size, neatly arranged, and normal in morphology.

3.2.2. Model Group. The structure of the lacrimal gland is not clear, the size of acinar and lacrimal gland epithelium is different, and the arrangement is disordered. Many acinar atrophy and fusion are formed and did the vacuoles.

3.2.3. 1.0 mg/ml Buddleja officinalis Maxim Eye Drops. The lacrimal gland structure is clear, and the acinar and lacrimal gland epithelium are uniform in size and loosely arranged, showing a small amount of acinar atrophy and vacuolization.

3.2.4. 1.5 mg/ml Buddleja officinalis Maxim Eye Drops. The lacrimal gland structure is clear, the acinar and lacrimal gland epithelium are uniform in size and arranged neatly, and a small number of vacuoles are formed.

Table 1: Comparison of SIT values at the same time of each group ($\overline{x} \pm S$).

Group	Before modeling	8 wk after modeling	14 d after medication	28 d after medication
A	15.50 ± 1.246	$16.08 \pm 0.975^{**}$	$17.00 \pm 0.862^{**}$	$18.42 \pm 0.596^{**\Delta\Delta}$
B	$13.83 \pm 1.492^{\blacktriangle}$	$2.92 \pm 0.499^{\blacktriangle\blacktriangle}$	$3.58 \pm 0.557^{\blacktriangle\blacktriangle}$	$2.42 \pm 0.583^{\blacktriangle\blacktriangle\Delta\Delta}$
C	$15.50 \pm 1.011^{\blacktriangle *}$	$3.42 \pm 0.583^{\blacktriangle\blacktriangle *}$	$6.75 \pm 0.808^{\blacktriangle\blacktriangle **}$	$8.92 \pm 0.398^{\blacktriangle\blacktriangle **\Delta}$
D	$16.67 \pm 1.378^{\blacktriangle *}$	$4.33 \pm 0.762^{\blacktriangle\blacktriangle *}$	$8.25 \pm 0.863^{\blacktriangle\blacktriangle **}$	$13.92 \pm 1.270^{\blacktriangle\blacktriangle **}$
E	$16.42 \pm 1.234^{\blacktriangle *}$	$3.42 \pm 0.514^{\blacktriangle\blacktriangle *}$	$9.75 \pm 1.162^{\blacktriangle\blacktriangle **}$	$14.50 \pm 1.138^{\blacktriangle\blacktriangle **\Delta}$
F	$15.67 \pm 1.047^{\blacktriangle *}$	$4.08 \pm 0.468^{\blacktriangle\blacktriangle *}$	$6.92 \pm 0.657^{\blacktriangle\blacktriangle **}$	$11.50 \pm 1.019^{\blacktriangle\blacktriangle **}$

Group A is the control group (male rabbit without any treatment); Group B is the dry eye model rabbit group; Group C is the dry eye rabbit model group treated with 1 mg/ml *Buddleja officinalis* Maxim eye drops; Group D is the dry eye rabbit model group treated with 1.5 mg/ml *Buddleja officinalis* Maxim eye drops; Group E is the dry eye rabbit model group treated with 3 mg/ml *Buddleja officinalis* Maxim eye drops; Group F is the dry eye rabbit group treated with testosterone. $^{\blacktriangle\blacktriangle}P < 0.01$ compared with Group A; $^{\blacktriangle}P > 0.05$ compared with Group A; $^{**}P < 0.01$ compared with Group B; $^{*}P > 0.05$ compared with Group B; $^{\Delta\Delta}P < 0.01$ compared with Group F; $^{\Delta}P > 0.05$ compared with Group F.

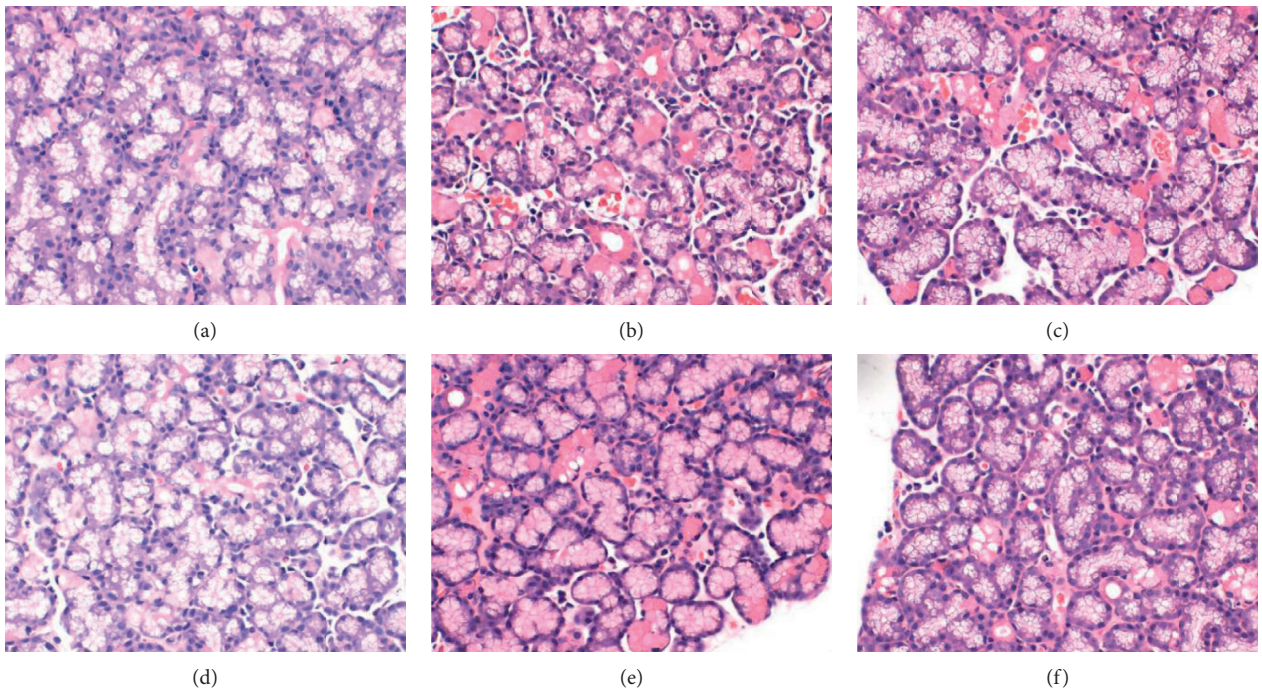


Figure 1: HE staining of the lacrimal gland (×400 times): (a) control group; (b) model group; (c) 1.0 mg/ml *Buddleja officinalis* Maxim eye drops; (d) 1.5 mg/ml *Buddleja officinalis* Maxim eye drops; (e) 3.0 mg/ml *Buddleja officinalis* Maxim eye drops; (f) testosterone group.

3.2.5. 3.0 mg/ml Buddleja officinalis Maxim Eye Drops. The lacrimal gland structure is clear, the acinar and lacrimal gland epithelium are uniform in size and neatly arranged, and the shape is normal, showing a little vacuole (Figure 1).

3.2.6. Testosterone Group. The lacrimal gland structure is clear, the acinar and lacrimal gland epithelium are uniform in size and neatly arranged, and the shape is normal, showing a little vacuole (Figure 2).

3.2.7. Immunohistochemical Method Was Used to Detect the Expression of Apoptotic Factors PI3K, Akt, and Caspase-9 in Lacrimal Gland of Male Rabbits. Immunohistochemistry was used to detect the expression of the apoptotic factor PI3K in the lacrimal gland of male rabbits. Compared with the testosterone group, there was no statistically significant difference between the control group and 1.0 mg/ml and the 1.5 mg/ml *Buddleja officinalis* Maxim eye drops group ($P > 0.05$). Compared with the control group, there was no significant difference between the 1.0 mg/ml and 1.5 mg/ml *Buddleja officinalis* Maxim eye drops and the testosterone group ($P > 0.05$).

About Akt, compared with the testosterone group, there was no statistically significant difference between the control group and the 3.0 mg/ml *Buddleja officinalis* Maxim eye drops group ($P > 0.05$). Compared with the control group, there was no significant difference between the 3.0 mg/ml *Buddleja officinalis* Maxim eye drops and the testosterone group ($P > 0.05$).

About caspase-9, compared with the testosterone group, there was no statistically significant difference between the control group and the 1.5 mg/ml and the 3.0 mg/ml *Buddleja officinalis* Maxim eye drops group ($P > 0.05$). Compared with the control group, there was no significant difference between the 1.5 mg/ml and the 3.0 mg/ml *Buddleja officinalis* Maxim eye drops and the testosterone group ($P > 0.05$) (Table 2; Figures 2 and 3).

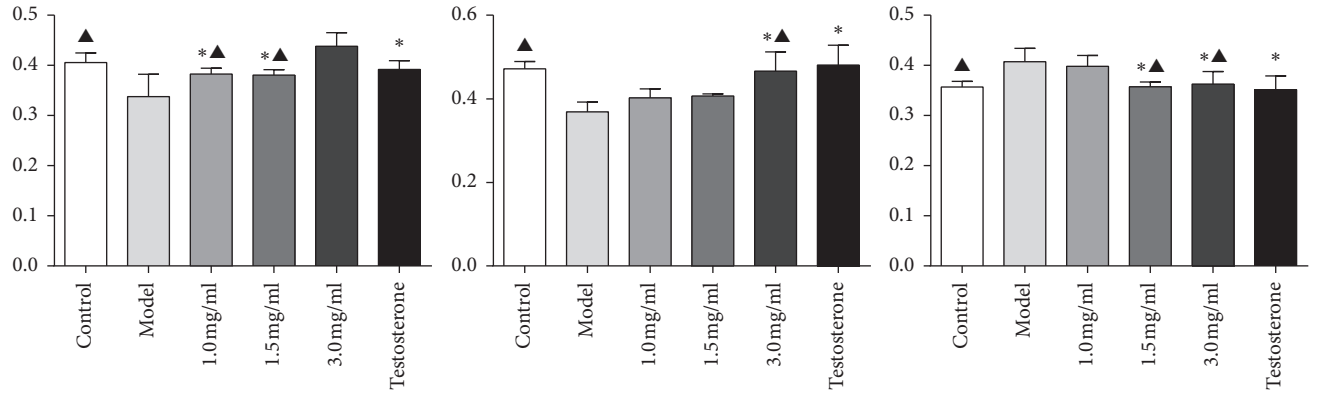
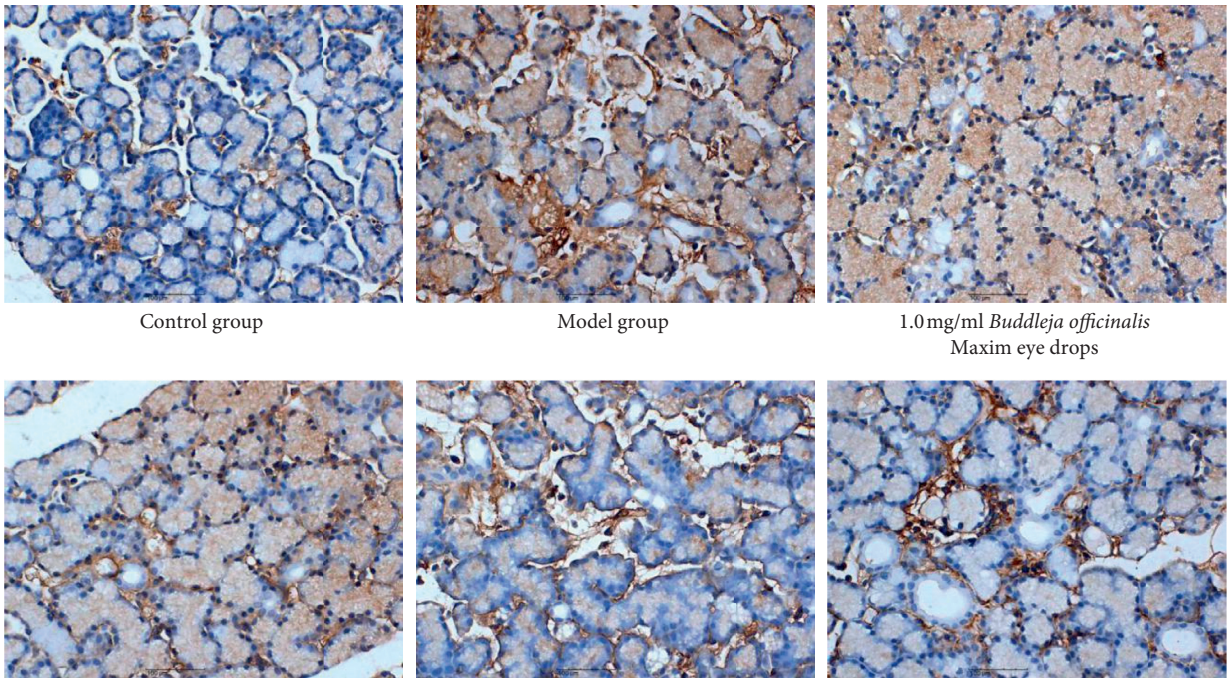


Figure 2: Comparison of average optical density values of (a) PI3K, (b) Akt, and (c) caspase-9.

Table 2: Comparison of average optical density values of PI3K, Akt, and caspase-9 ($\overline{x} \pm SD$).

Group	n	PI3K	Akt	Caspase-9
A	12	$0.4055 \pm 0.0191^{\blacktriangle}$	$0.4715 \pm 0.0177^{\blacktriangle}$	$0.3570 \pm 0.0113^{\blacktriangle}$
B	12	0.3379 ± 0.0445	0.3685 ± 0.0235	0.4074 ± 0.0264
C	12	$0.3828 \pm 0.0115^{\blacktriangle *}$	0.4023 ± 0.0214	0.3980 ± 0.0219
D	10	$0.3807 \pm 0.0106^{\blacktriangle *}$	0.4067 ± 0.0053	$0.3572 \pm 0.0097^{\blacktriangle *}$
E	12	0.4380 ± 0.0273	$0.4662 \pm 0.0461^{\blacktriangle *}$	$0.3626 \pm 0.0250^{\blacktriangle *}$
F	10	$0.3918 \pm 0.0177^{*}$	$0.4807 \pm 0.0473^{*}$	$0.3516 \pm 0.0273^{*}$

$^{*}P > 0.05$ compared with Group A; $^{\blacktriangle}P > 0.05$ compared with Group F.



Control group

Model group

1.0 mg/ml *Buddleja officinalis* Maxim eye drops

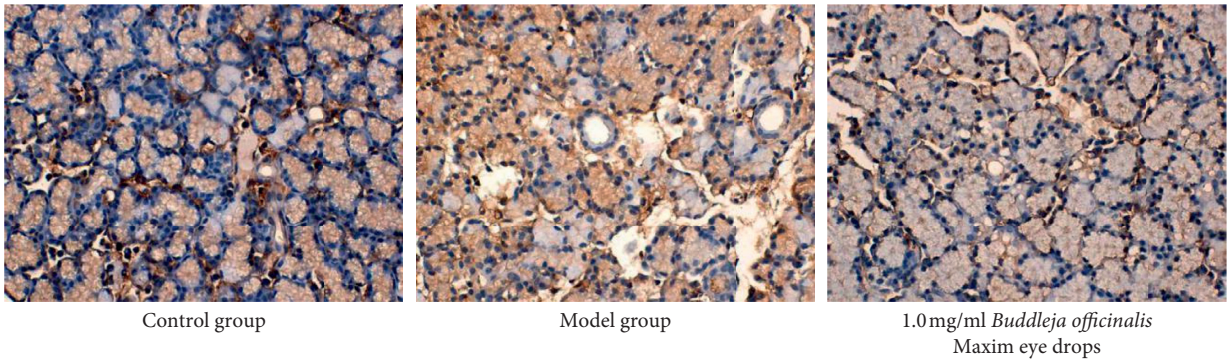
1.5 mg/ml *Buddleja officinalis* Maxim eye drops

3.0 mg/ml *Buddleja officinalis* Maxim eye drops

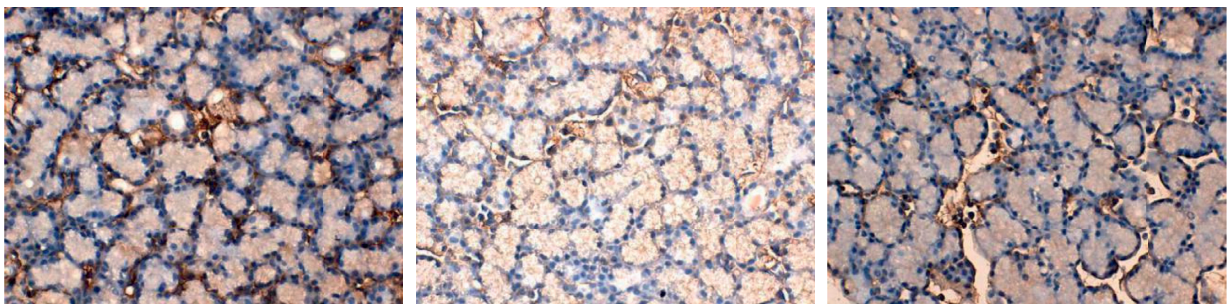
Testosterone group

(a)

Figure 3: Continued.

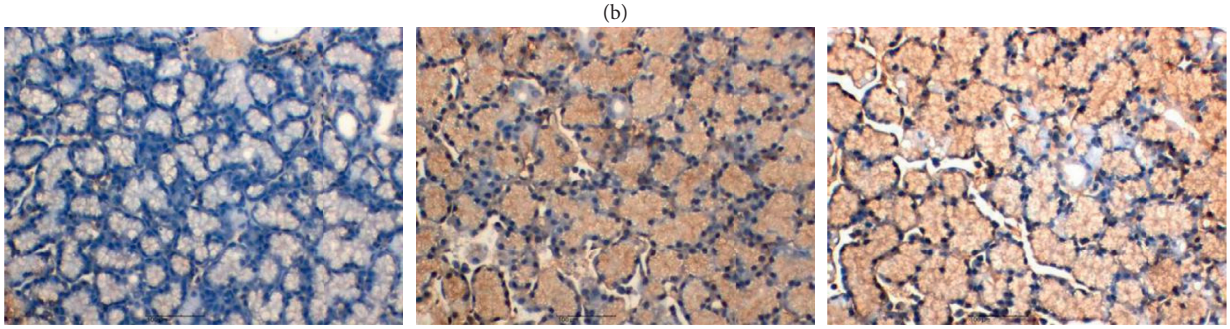


Control group | Model group | 1.0 mg/ml *Buddleja officinalis* Maxim eye drops

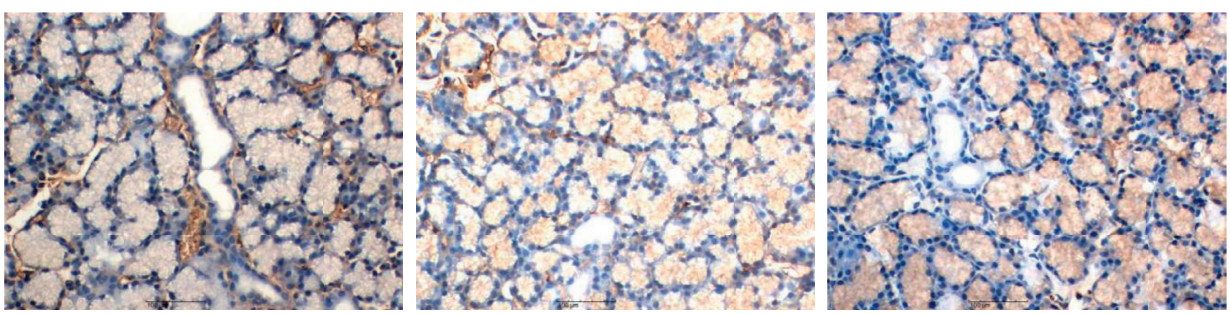


1.5 mg/ml *Buddleja officinalis* Maxim eye drops | 3.0 mg/ml *Buddleja officinalis* Maxim eye drops | Testosterone group

(b)



Control group | Model group | 1.0 mg/ml *Buddleja officinalis* Maxim eye drops



1.5 mg/ml *Buddleja officinalis* Maxim eye drops | 3.0 mg/ml *Buddleja officinalis* Maxim eye drops | Testosterone group

(c)

Figure 3: Immunohistochemical observation of (a) PI3K, (b) Akt, and (c) caspase-9 protein expression in the lacrimal gland (×400 times).

4. Discussion

Our study found that the use of *Buddleja officinalis* Maxim extract as a dry eye drop improved the lacrimal gland histology and cellular makeup and that this matched the effect of topical testosterone. The average optical density values of PI3K, Akt, and caspase-9 protein in the lacrimal gland tissue of the 3.0 mg/ml *Buddleja officinalis* Maxim eye drops group were significantly different from those in the model group ($P < 0.01$) and like the testosterone control group and the control group ($P > 0.05$).

Dry eye caused by decreased sex hormone levels is a key concern in the dry eye field in recent years. The 2017 TFOS Dry Eye Working Group devoted a discussion on the relationship between biological sex, gender, hormones, and dry eye, and the importance of which is the second only to pathology and treatment [2]. Among them, androgen is a very important position in the regulation of the epidermis and attachment of the eye, which mediates many sex-related differences in the tissue [11]. Related studies have confirmed that the eye is one of the main target organs of sex hormones [12]. Sex hormone receptors (including androgens, estrogens, and progesterone) are widely present in the tear function unit of human, mouse, and rabbit [11]. Among them are the lacrimal gland, meibomian gland, cornea, and conjunctiva. As a target organ of androgen, the lacrimal gland is regulated by androgen levels in its synthesis and secretion [13–15].

In this study, the high-performance liquid chromatography was used to extract the *Buddleja officinalis* Maxim and found that the total flavonoids and phenylpropanoids were the main components of the *Buddleja officinalis* Maxim, and then we made it to eye drops. Eye drop is one of the most commonly used dosage forms for ophthalmic treatment, and the *Buddleja officinalis* Maxim may access to the lacrimal gland through tissue infiltration. Therefore, *Buddleja officinalis* Maxim Eye Drops has a wide range of the application value, but its preparation process and optimal therapeutic concentration need to be further explored. The effective components of *Buddleja officinalis* Maxim also need to be further explored. And in our research, we focused on the screening of drug concentrations, and ignoring the design of the placebo-treated group and the sham-operated group, the absence of placebo-treated and sham-operated groups of rabbits was a limitation in our study, and we will correct it in the future experiments to increase the rigour of the experiment.

Data Availability

The data used to support the findings of this study are available from the article, and the data are available and can be shared with the publisher upon request.

Conflicts of Interest

The data in tables and figures used to support the findings of this study are included within the article.

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Research Article

The Effects of Diabetic Duration on Lacrimal Functional Unit in Patients with Type II Diabetes

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Purpose. To observe ocular surface changes in Type II diabetic patients with different disease durations and to understand the correlations between clinical parameters and diabetic durations. *Methods*. In this cross-sectional, prospective study, 51 healthy controls and 91 patients with Type II diabetes were enrolled. The diabetics were divided into 3 subgroups according to the disease duration, including duration <10 y group, 10 to 20 y group, and ≥21 y group. All subjects underwent clinical ocular examinations, including lipid layer thickness (LLT), blinking rate, tear meniscus height (TMH), noninvasive tear film break-up time (NI-BUT), meibography, superficial punctate keratopathy (SPK) scoring, corneal sensitivity, and Schirmer I test. They were also evaluated using the standard patient evaluation of eye dryness (SPEED) questionnaire. *Results*. SPEED score, meiboscore, SPK score, LLT, Schirmer I test, and corneal sensitivity differed significantly between the diabetic and healthy control groups. Further, SPEED score, Schirmer I test, corneal sensitivity, meiboscore, and blink rate significantly differed among the 3 diabetic subgroups and the control group. In diabetics, the SPEED score correlated with the SPK score, blink rate, TMH, and LLT; NI-BUT with TMH, LLT, and blink rate; TMH with the SPK score; Schirmer I test with the SPK score; and corneal sensitivity with the meiboscore. More importantly, the Schirmer I test, corneal sensitivity, and SPEED score negatively correlated with diabetic duration. *Conclusion*. Diabetic duration is an important factor that affects functions of the lacrimal functional unit in patients with Type II diabetes. The trends of changes in the ocular parameters vary along the course of diabetes.

1. Introduction

The lacrimal function unit (LFU) is composed of ocular surface (cornea, conjunctiva, and meibomian glands), lacrimal gland, and a neural network that connects them. It protects lipid, aqueous, and mucin layers of tear film and maintains normal function of ocular surface [1]. If any component of the LFU was damaged, it could lead to reduced tear production, abnormalities in blinking, and changes in tear film composition [2]. The individuals with long-standing hyperglycemia are at an increased risk of developing LFU dysfunction. Clinical studies have demonstrated that diabetic patients were more susceptible to ocular surface disorders than healthy subjects. For instance, keratoepitheliopathy was evident [3–7]; corneal sensitivity [7–11], quantity, and quality of tear secretion [3, 7, 11–14] were reduced in diabetic patients; moreover, the alterations in tear composition [15–18] were also detected in the diabetics. However, the factors contributing to the LFU dysfunction are not clear, and they might include the patient's general condition such as age and gender [5, 19–21], metabolic control [3, 12, 22, 23], duration of diabetes [20, 21], and occurrence of diabetic microvascular complications [5, 8, 22, 23].

The diabetic duration is considered one of the most important risk factors for retinopathy [21, 24, 25], yet its role in the LFU dysfunction remains controversial. Several studies reported no relationship between duration of diabetes and tear functions [3, 12, 13, 22, 23]; whereas others indicated higher prevalence of dry eye syndrome in the

patients with longer duration of diabetes [21]; further, a correlation was found between diabetic duration and deterioration of ocular surface clinical parameters [20].

Therefore, this study sought to examine and compare the clinical parameters of ocular surface in the diabetic patients divided into 3 subgroups according to duration of the disease. The correlation between the diabetic duration and clinical ocular surface parameters was also assessed.

2. Materials and Methods

2.1. Patients. One hundred eight-two eyes of 91 patients with Type II diabetes (diabetic group) and 102 eyes of 51 normal individuals (control group) were enrolled in the current study at Tianjin Medical University Eye Hospital (Tianjin, China) between July and September in 2017. Due to the relatively small number of middle-aged and young people suffering from Type II diabetes and the fact that the duration of diabetes in the middle-aged and young diabetic patients is usually not long enough to reflect the influence of the disease duration on ocular surface functions, the diabetic patients with an average age of 65.43 ± 6.31 y (range 55 to 80 y) were recruited, matching the control group (average age 64.35 ± 5.66 y). Moreover, the diabetic patients should have dry eye symptoms (SPEED ≥ 1), should not resort to ocular medication or surgery within the past 3 m, and should be without ocular injury and diseases including infection, allergy, glaucoma, and autoimmune diseases, and with no history of systemic diseases or administration of systemic medications, such as sex hormone replacement, parasympathomimetics, and parasympatholytics, that may affect tear production or quality. Diabetes was confirmed in all patients by the Department of Internal Medicine; glycosylated hemoglobin levels in these patients were less than 7.8%. The diabetics were further divided into 3 subgroups according to the disease duration: duration <10 y group, duration 10 to 20 y group, and duration ≥21 y group. Retinal status was evaluated by indirect ophthalmoscopy exam and fluorescein angiography, and no PDR was detected in the patients according to the early treatment diabetic retinopathy study criteria [26].

Written informed consent was obtained from all the participants enrolled in this study after a thorough explanation of the study objective and methods. All procedures of this study were approved by the ethical committee in Tianjin Medical University Eye Hospital (Ethical no.: 2017KY (L)-18) and in accordance with the tenets of the Declaration of Helsinki. This study was registered at Chinese Clinical Trial Registry (registration number: ChiCTR-ROC-17011707).

2.2. Questionnaire. All patients were required to fill out a questionnaire (standard patient evaluation of eye dryness (SPEED)) [27] for assessing ocular surface symptoms prior to routine ophthalmic examinations. The scores on the questionnaire ranged from 0 to 28 according to the severity of patients' symptoms. The symptoms included dryness, grittiness or scratchiness, soreness or irritation, burning or watering, and eye fatigue. The frequency of the symptoms was graded as never (0), sometimes (1), often (2), and constantly (3). The subjective sensation of the symptoms was categorized as no problems (0), tolerable (1), uncomfortable (2), bothersome (3), and intolerable (4).

2.3. Lipid Layer Thickness and Blink Assessment. The LipiView interferometer (TearScience Inc., Morrisville, NC) was used to capture a 20 s video of interference pattern of the subject's tear film. In addition to counting the subject's total and partial blinks, the interferometer converts the specific interference colors into the values of lipid layer thickness (LLT) [28, 29].

2.4. Tear Meniscus Height, Noninvasive Tear Break-Up Time, and Meibography. The Keratograph 5M (Oculus GmbH, Wetzlar, Germany) equipped with a modified tear film-scanning function was used to measure tear meniscus height (TMH) by capturing the lower tear film meniscus images and detect noninvasive tear film break-up time (NI-BUT) as described previously [30]. Furthermore, the subject's upper and lower eyelids were everted, and the high-contrast image of meibomian glands (MGs) was acquired under an infrared meibography model [31]. The MG dropout area was quantified by a meiboscore (grade 0, no dropout; grade 1, <33% dropout; grade 2, 33 to 67% dropout; and grade 3, >67% dropout), and the scores from upper and lower eyelids were added (total meiboscore, range 0~6) to reflect MG dropout of the eye [32].

2.5. Superficial Punctate Keratopathy Score. The severity of corneal surface damage was evaluated by staining the cornea with fluorescein; both the staining area and staining density were scored from 0 to 3 as described previously [4, 33]. The specific criteria are listed in Table 1. The product of both scores was calculated, termed superficial punctuate keratopathy (SPK), and used as an index for the damage of corneal surface.

2.6. Corneal Sensitivity. A Cochet–Bonnet aesthesiometer was used to examine corneal sensitivity as described elsewhere [34]. The tip of a fully extended nylon filament was applied to the central cornea at a perpendicular angle, and the thread length was recorded when the subject felt its presence.

2.7. Schirmer I Test. Total tear secretion was measured without anesthesia by placing a standardized Schirmer strip into accus conjunctivae at lateral 1/3 of lower lid for 5 min with eyes closed gently, and then the length of the wet strip was recorded.

2.8. Statistical Analysis. Statistical analyses were performed using Statistical Program for Social Sciences 20.0 (IBM SPSS Inc., New York, NY, USA). All data were expressed as mean ± SEM. The data were examined using the D'Agostino and Pearson omnibus normality test. The data with a

Table 1: Classification of severity in corneal epithelial lesions.

Area: corneal surface area	Density: density of damaged lesions
A0: no punctate staining	D0: no punctate staining
A1: less than one-thirds	D1: sparse density
A2: one third to two-thirds	D2: moderate density
A3: more than two-thirds	D3: high density with overlapping lesions

Gaussian distribution were further examined by the Levene test to confirm homogeneity of variance. The differences among the diabetic duration subgroups and the healthy controls were analyzed by one-way ANOVA followed by a Tukey post hoc. For the data with nonparametric distribution, the differences among groups were analyzed by the Kruskal–Wallis test followed by Dunn's post hoc. The associations between the parameters were analyzed by Spearman's correlation analysis. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. General Condition. The gender ($\chi^2 = 1.015$, $P = 0.314$) and age ($t = -1.428$, $P = 0.154$) did not differ significantly between the diabetes and control groups (Table 2). Moreover, no significant difference was found in gender ($\chi^2 = 3.854$, $P = 0.278$), age ($F = 0.881$, $P = 0.452$) or percentage of HbA1c ($F = 1.158$, $P = 0.316$) among the patients subgrouped according to the diabetic duration and the control group (Table 2). The prevalence of DR in total diabetic patients was 70.9%. The DR incidence was 25% in the patients with diabetes less than 10 y, 85.9% in those with diabetes 10–20 y, and 100% in those with the disease for more than 21 y, suggesting a significantly increased DR incidence as the diabetic duration prolongs ($\chi^2 = 87.084$, $P \leq 0.001$; Table 2).

3.2. Comparison of Ocular Surface Parameters between Diabetics and Healthy Controls. There were no significant differences in blink frequency, NI-BUT, and TMH values between the diabetes and control groups (Table 3, Figure 1). The SPEED score ($Z = -3.600$, $P \leq 0.001$) (Figure 1(a)), meiboscore ($t = -4.003$, $P \leq 0.001$) (Figure 1(c)), and SPK score ($Z = -2.463$, $P = 0.014$) (Figure 1(h)) in the diabetic group were significantly higher than those in the control group. In addition, LLT values ($t = -2.018$, $P = 0.045$) (Figure 1(d)), Schirmer I test results ($Z = -1.991$, $P = 0.046$) (Figure 1(e)), and corneal sensitivity ($t = -4.100$, $P \leq 0.001$) (Figure 1(g)) were significantly decreased in the diabetic group as compared to the control group.

3.3. Comparison for Ocular Surface Parameters among the Diabetic Duration Subgroups and Control Group. The SPEED score ($H = 16.630$, $P = 0.001$), Schirmer I test result ($H = 14.164$, $P = 0.003$), corneal sensitivity ($F = 11.344$, $P \leq 0.001$), meiboscore ($F = 4.950$, $P = 0.002$), and blink rate ($H = 10.232$, $P = 0.017$) were significantly different among the diabetic subgroups and the healthy control group (Table 3, Figure 2). Other parameters, such as SPK score, NI-BUT, TMH, and LLT values, did not exhibit significant differences among these groups (Table 3, Figure 2). The SPEED score (Figure 2(a)) in the control group was significantly lower than those in the subgroups with diabetic duration <10 y ($Z = -3.912$, $P \leq 0.001$) and 10 to 20 y ($Z = -2.510$, $P = 0.012$). The meiboscore was significantly higher in the subgroups with diabetic duration <10 y ($t = -2.166$, $P = 0.033$), duration 10 to 20 y ($t = -3.675$, $P \leq 0.001$), and duration ≥21 y ($t = -2.481$, $P = 0.015$) than that in the healthy controls (Figure 2(c)). The LLT value in the diabetic subgroup with duration ≥21 y was significantly reduced as compared with the controls ($t = -2.949$, $P = 0.004$, Figure 2(d)). The value of the Schirmer I test in the control group was significantly higher than those in the subgroups with diabetic duration 10 to 20 y ($Z = -2.773$, $P = 0.006$) and duration ≥21 y ($Z = -2.053$, $P = 0.040$, Figure 2(e)). Also, the subgroup with diabetic duration <10 y showed a higher Schirmer I test value than those in the subgroups with duration 10 to 20 y ($Z = -3.000$, $P = 0.003$) and duration ≥21 y ($Z = -2.674$, $P = 0.007$, Figure 2(e)). As for corneal sensitivity, the recorded length of nylon filament in the control group was significantly longer, indicative of higher corneal sensitivity, than those in the subgroups with duration 10 to 20 y ($t = 2.716$, $P = 0.007$) and ≥21 y ($t = 4.640$, $P \leq 0.001$). Moreover, corneal sensitivity in the subgroup with duration ≥21 y was significantly deteriorated as compared to the subgroups with duration <10 y ($t = 3.605$, $P = 0.001$) and duration 10 to 20 y ($t = 2.640$, $P = 0.010$) (Figure 2(g)). The SPK score was significantly increased in the subgroup with duration <10 y as compared with healthy controls ($Z = -2.463$, $P = 0.014$), indicating the diabetes-induced damage on ocular surface (Figure 2(h)). The diabetic subgroup with duration of 10 to 20 y had greater blink frequency than the healthy control group ($Z = -3.044$, $P = 0.002$), the diabetic subgroups with the duration <10 y ($Z = -2.127$, $P = 0.033$), and duration ≥21 y ($Z = -2.203$, $P = 0.027$) (Figure 2(i)).

3.4. Correlations of the Ocular Surface Parameters in Diabetic Groups. In the diabetic patients, the score of SPEED was positively correlated with the SPK score ($r = 0.300$, $P \leq 0.001$) and blink rate ($r = 0.146$, $P = 0.050$) and negatively correlated with TMH ($r = -0.151$, $P = 0.042$) and LLT values ($r = -0.286$, $P \leq 0.001$) (Figure 3). In addition, corneal sensitivity was positively correlated with the meiboscore ($r = 0.153$, $P = 0.040$) and barely correlated with the SPEED score ($r = 0.144$, $P = 0.052$) (Figure 3). There were also positive correlations between NI-BUT and TMH ($r = 0.167$, $P = 0.024$) as well as between NI-BUT and LLT values ($r = 0.160$, $P = 0.031$) (Figure 3). On the other hand, negative correlations were found between NI-BUT and blink frequency ($r = -0.193$, $P = 0.009$), between TMH and SPK score ($r = -0.165$, $P = 0.026$), as well as between the Schirmer I test value and SPK score ($r = -0.195$, $P = 0.008$) (Figure 3).

Table 2: Demographics of diabetic subgroups and control group.

Demographics	Controls	Diabetic patients			
		<10 y	10~20 y	≥21 y	Total
Subject (*n*)	51	28	39	24	91
M : F ratio	18 : 33	11 : 17	18 : 21	14 : 10	40 : 51
Age (y)	64.35 ± 5.66	64.93 ± 6.03	65.54 ± 6.41	65.83 ± 6.57	65.43 ± 6.31
HbA1c (%)	—	6.94 ± 0.43	7.00 ± 0.51	6.87 ± 0.53	6.95 ± 0.49
Duration (y)	0	4.18 ± 2.02	13.67 ± 2.46	24.22 ± 4.81	13.58 ± 8.27
DR (no. of eyes)	0	56	78	48	182
NDR (*n* (%))	0	42 (75)	11 (14.1)	0	53 (29.1)
NPDR (*n* (%))	0	14 (25)	67 (85.9)	48 (100)	129 (70.9)
PDR (%)	0	0	0	0	0

Note: duration, duration of diabetes; DR, diabetic retinopathy; NDR, no diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

Table 3: Ocular surface clinical parameters in diabetic patients and normal controls.

Parameters	Controls	Diabetic patients			
		<10 y	10~20 y	≥21 y	Total
SPEED	5.24 ± 3.04	7.25 ± 2.99	6.56 ± 3.48	6.21 ± 3.10	6.68 ± 3.24
LLT (nm)	75.96 ± 19.79	70.71 ± 20.15	73.69 ± 22.07	66.71 ± 16.98	71.04 ± 20.46
Blinks (times)	4.43 ± 3.57	4.73 ± 4.05	6.23 ± 4.25	4.81 ± 4.28	5.40 ± 4.24
NI-BUT (sec)	9.97 ± 5.19	10.22 ± 6.13	10.02 ± 6.33	8.68 ± 4.85	9.73 ± 5.91
TMH (mm)	0.22 ± 0.86	0.24 ± 0.75	0.24 ± 0.11	0.23 ± 0.81	0.24 ± 0.10
Meiboscore	2.46 ± 0.86	2.84 ± 1.14	3.03 ± 1.13	2.92 ± 1.13	2.94 ± 1.13
SPK score	0.32 ± 0.73	0.64 ± 1.09	0.44 ± 0.69	0.44 ± 0.68	0.50 ± 0.83
Sensitivity	58.19 ± 4.15	57.41 ± 5.04	56.13 ± 5.62	52.81 ± 7.50	55.65 ± 6.24
Schirmer (mm)	9.25 ± 8.07	9.23 ± 7.18	5.73 ± 4.89	5.56 ± 4.20	6.76 ± 5.76

Note. SPEED, standard patient evaluation of eye dryness; LLT, lipid layer thickness; NI-BUT, noninvasive tear film break-up time; TMH, tear meniscus height; meiboscore, total percentage of meibomian gland dropout area in upper and lowers eyelids; SPK score, superficial punctate keratopathy score; sensitivity, corneal sensitivity; Schirmer, Schirmer I test (total tear secretion).

3.5. Correlations between Ocular Surface Parameters and Duration of Diabetes. In the total diabetic group, the Schirmer I test ($r = -0.268$, $P \leq 0.001$), corneal sensitivity ($r = -0.336$, $P \leq 0.001$), and SPEED score ($r = -0.171$, $P = 0.021$) exhibited negative correlations with duration of diabetes (Figure 4).

4. Discussion

Diabetes mellitus is a systemic disease characterized by chronic hyperglycemia and dysregulated metabolism and may lead to LFU dysfunctions through different mechanisms. Patients with diabetes have demonstrated structural, metabolic, and functional abnormalities in the cornea and conjunctiva, which subsequently increase the risk of developing diabetic complications in ocular surface [3–6, 12, 23].

It has been proposed that as the duration of diabetes increases, the risk of developing proliferative diabetic retinopathy [24, 25] and diabetic neuropathy [8–11] increases dramatically; therefore, we would expect the ocular surface parameters measured in this study to become significantly exacerbated as diabetes persists. However, this is not necessarily true based on our results, as varying degrees of deterioration in the LFU, including tear film, ocular surface function, and corneal sensation, were detected in the patients afflicted by diabetes for different periods time (Figure 2, Table 3). The duration of diabetes was only negatively correlated with the SPEED score, Schirmer I test, and corneal sensitivity in the diabetes group (Figure 4, Table 3).

Apart from the diabetic subgroup with the disease duration ≥21 y, we found that the SPEED scores in other diabetes subgroups were significantly greater than the healthy controls (Figure 1(a)). Further, the SPEED score exhibits a trendy correlation with the corneal sensitivity (Figure 3) and a significant negative correlation with the diabetic duration (Figure 4(a)). These results suggest that subjective symptoms became exacerbated in early diabetic patients and then attenuated as the disease persisted; this may be due to the blunted corneal sensitivity caused by peripheral neuropathy during long-term diabetes.

Abnormalities in the quantity and quality in tear secretion have been reported in diabetes, but the results remain controversial. In contrast to the results of other studies, the data in this study showed that the difference in NI-BUT did not reach a statistical significance between diabetes and control group (Figure 1(b)). This was probably because the distinct apparatus and calculation method were used to measure the tear film BUT in the current study; the difference between the diabetics and normal controls might become less dramatic [28]. However, the NI-BUT in this study did exhibit a trendy decrease as diabetes persisted (Figure 2(b), Table 3), which is consistent with the results of previous studies [3, 7, 12–14, 19].

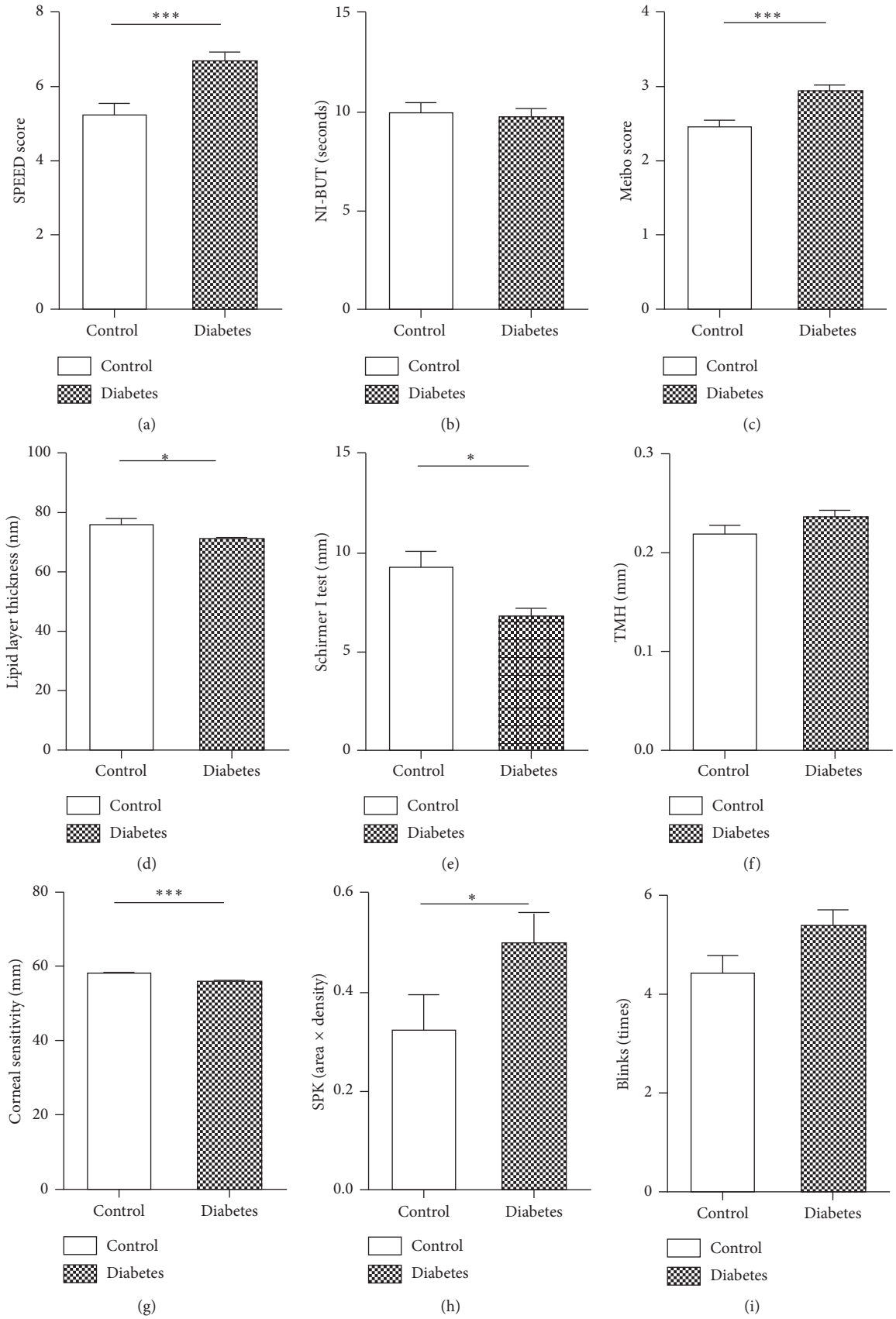
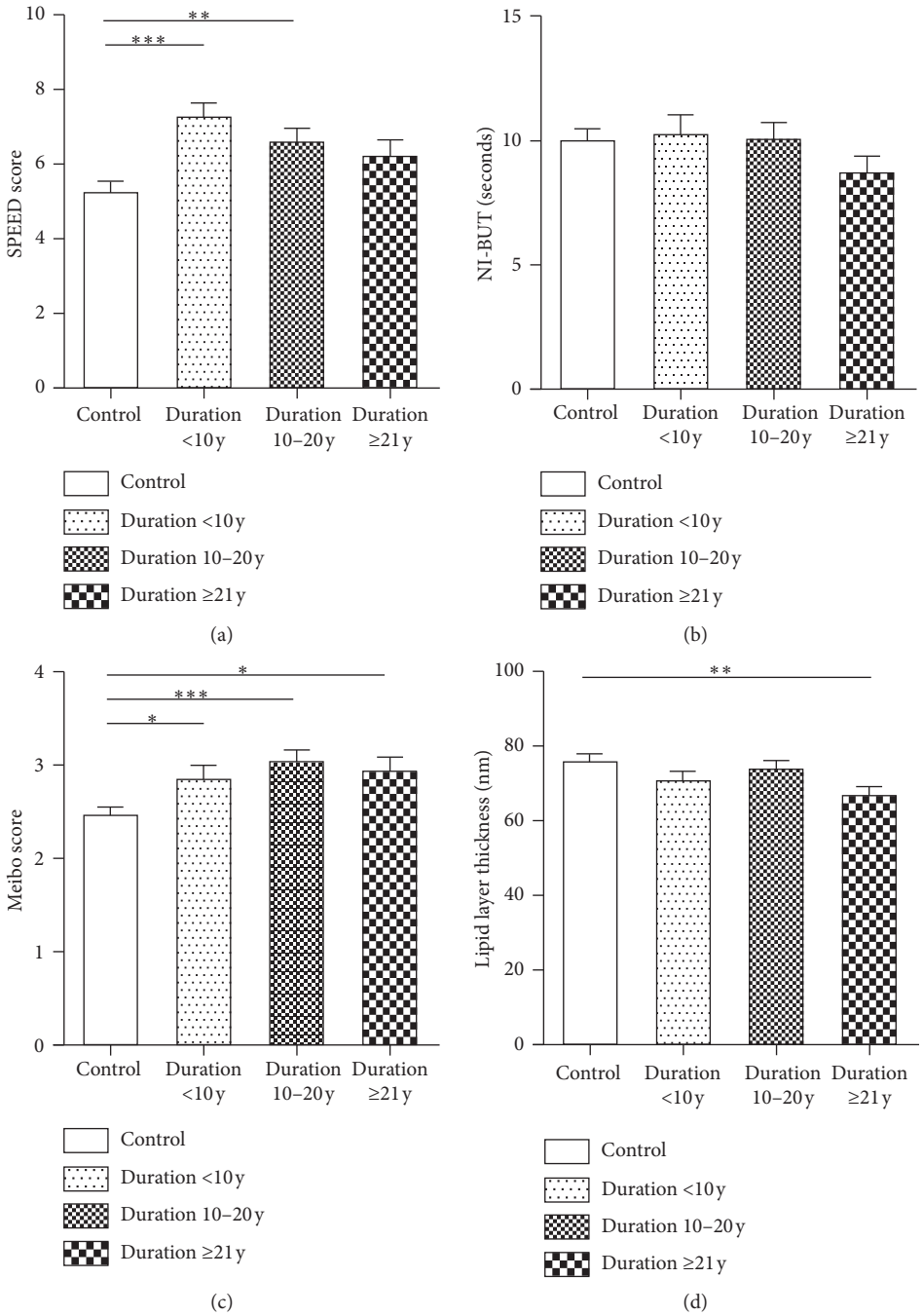


Figure 1: Comparison of ocular surface parameters between diabetic patients and healthy controls. The SPEED score (a), meiboscore (c), lipid layer thickness values (d), Schirmer I results (e), corneal sensitivity (g), and SPK score (h) were significantly different between the diabetic and the healthy control group. The differences in NI-BUT (b), TMH (f), and blinks (i) did not reach statistical significance between the diabetic and control groups ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P \leq 0.001$).



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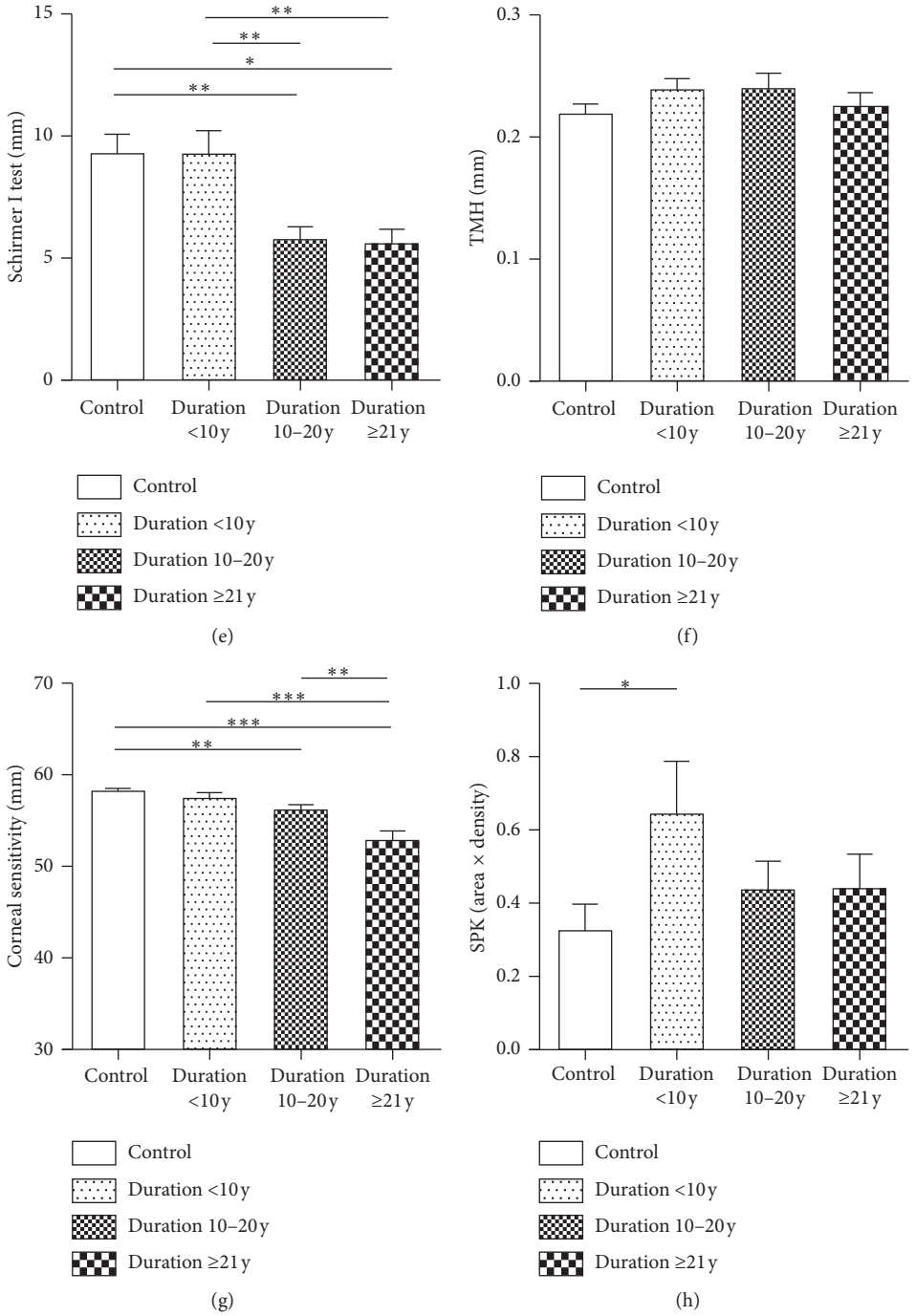

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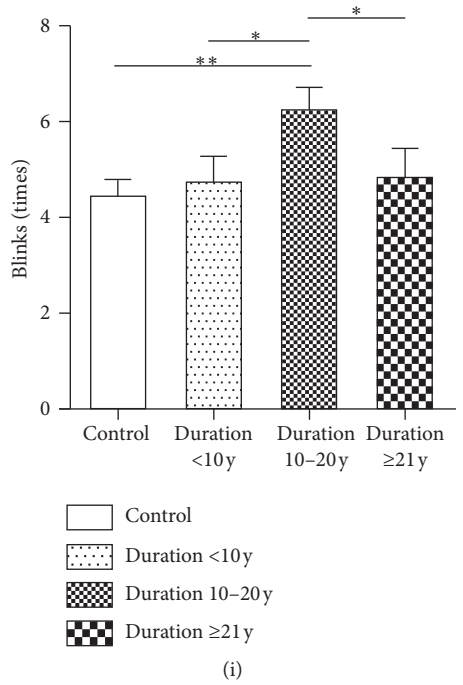

Figure 2: Comparisons of ocular surface parameters among the diabetic duration subgroups and the healthy control group. The SPEED score (a), NI-BUT (b), meiboscore (c), lipid layer thickness (d), Schirmer I test result (e), TMH (f), corneal sensitivity (g), SPK score (h), and blinks (i) were compared among the diabetic subgroups with different disease duration and the healthy control group ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P \leq 0.001$).

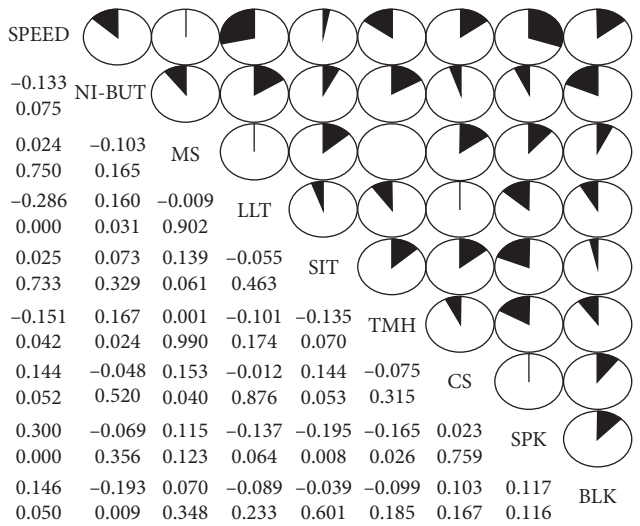

Figure 3: A correlogram illustrates the strength of correlations between ocular surface clinical parameters in the diabetic patients. In the pie graphs, the clockwise direction indicates a positive correlation and counterclockwise direction a negative correlation. For the numbers on the left of the pie graph, the upper one indicates the correlation coefficient and the lower one the P value (SPEED, standard patient evaluation of eye dryness; LLT, lipid layer thickness; NI-BUT, noninvasive tear film break-up time; TMH, tear meniscus height; MS, total percentage of meibomian gland dropout area in upper and lowers eyelids; SPK, superficial punctate keratopathy score; CS, corneal sensitivity; SIT, Schirmer I Test (total tear secretion)).

The MGs are large sebaceous glands that are innervated by parasympathetic fibers [35] and produce meibum, a major source of lipid in the tear film [36]. It is generally accepted that diabetic patients are more susceptible to neuropathy [10, 23], as well as blepharitis and recurrent styes resulting from infected sebaceous glands [37, 38]. Indeed, we found, in the current study, that the MG dropout is more severe in all the diabetic groups than the healthy controls (Figure 2(c)) and a positive correlation between MG dropout and corneal sensitivity (Figure 3). Furthermore, the LLT in diabetics was significantly deceased when compared to the controls (Figure 1(d)). Whereas a compromised lipid layer could, in turn, cause excessive evaporation of tear film [39]. Therefore, we speculate that diabetic neuropathy, repeated infection and inflammation, might contribute to obstruction in MG orifices, MG atrophy, and dropout in the patients with a long history of diabetes.

Besides the tear film instability and excessive evaporation, the diminished tear secretion was also observed in diabetic patients [3, 7, 12–14, 19]. Such reduced tear secretion has been considered to be caused by the decreased reflex tear secretion, which is positively correlated to corneal sensation [8, 14]. In our study, the decreased total tear secretion was observed in all diabetes groups, particularly in the subgroups with duration 10 to 20 y and duration ≥21 y when compared to the controls (Figure 2(e)). Moreover, we also found decreased corneal sensitivity in these two diabetic subgroups and a trendy positive correlation between total

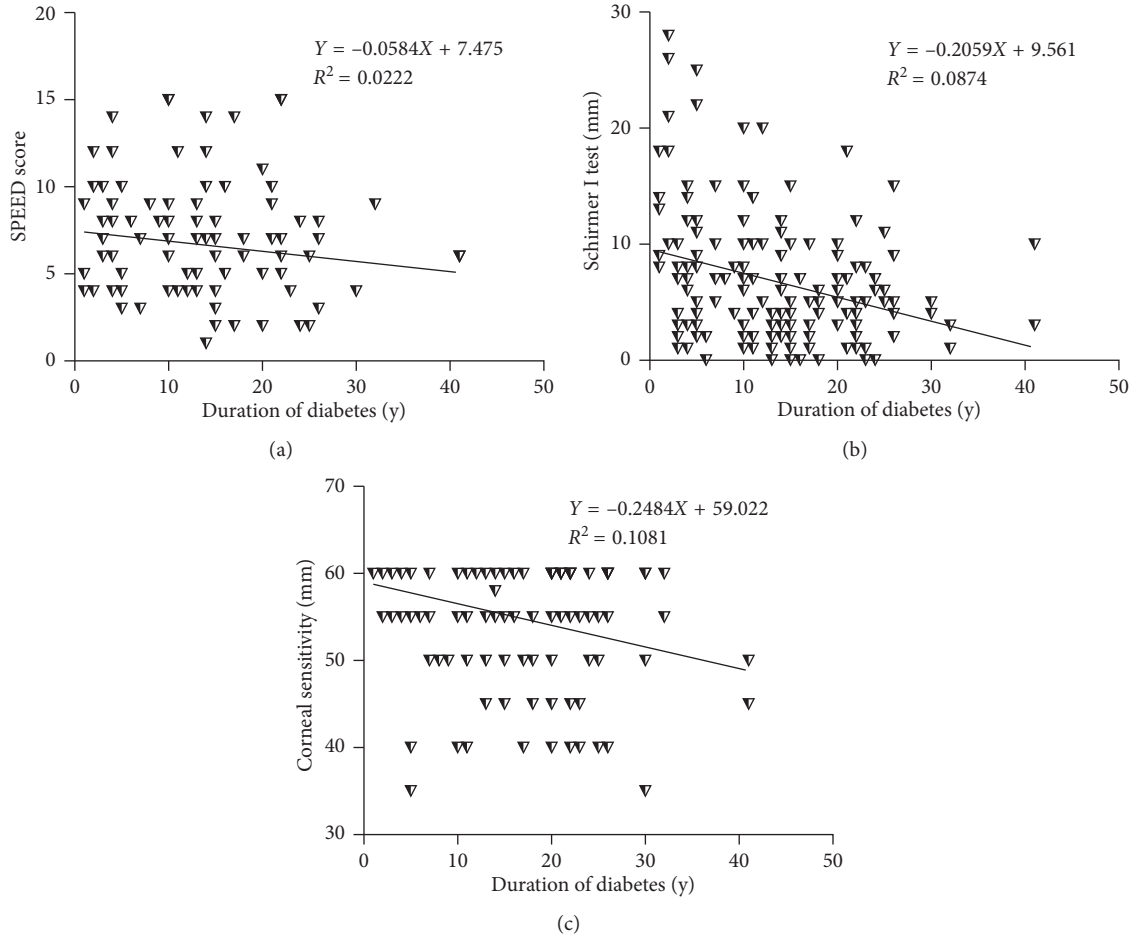

Figure 4: Correlations between ocular surface parameters and duration of diabetes. In the total diabetic group, the SPEED score (a), Schirmer I test (b), and corneal sensitivity (c) were negatively correlated with duration of diabetes.

tear secretion and corneal sensitivity (Figure 3). Furthermore, the total tear secretion is negatively correlated with the duration of diabetes (Figure 4(b)). These results suggest that the reduced total tear secretion may be caused, at least in part, by the impaired reflex tear secretion during long-term diabetes.

Diabetic neuropathy is one of the most common long-term complications of diabetes. As revealed by our result and previous studies [3, 7, 9–12, 19], diabetic patients have decreased corneal sensitivity. Furthermore, our finding revealed the significantly decreased corneal sensitivity in the diabetic subgroups with duration 10 to 20 y and duration ≥21 y as compared with controls (Figure 2(g)), suggesting that the symptoms of diabetic neuropathy often occur after 10 y of the disease onset. In addition, the negative correlation between the duration of diabetes and corneal sensitivity (Figure 4(c)) suggests that long-term hyperglycemia and metabolic syndrome in diabetes may deteriorate corneal sensation. As mentioned above, the deteriorated corneal sensitivity in diabetes leads to declined reflex tear secretion [8]. Moreover, the neurodegeneration in conjunctiva may result in abnormal secretion of mucin proteins from goblet cells, further compromising the quality and stability of tear film [40]. Thirdly, diabetic peripheral neuropathy in ocular surface can cause malnutrition and metabolic abnormalities in cornea, which consequently induces refractory corneal epithelial ulcer and erosion [41]. These factors may form a vicious cycle, exacerbating LFU dysfunction and ocular surface damage as diabetes persists.

Blinking plays an important role in maintaining the stability of tear film and is related to psychological and/or several systemic diseases [4, 20, 42]. In this study, we observed the increased blinking frequencies in all diabetes groups, but only the subgroup with diabetic duration of 10 to 20 y reached statistical significance as compared to the healthy controls (Figure 2(i)). This result was surprising, as one would expect the diabetics to have reduced blinking frequency as a result of the impaired corneal sensation. However, the ocular surface conditions in early diabetes, such as the reduced tear secretion, the unstable tear film caused by disruption in the lipid layer and paucity of mucin proteins, as well as the sterile or nonsterile inflammation, may still boost blinking frequency via the blunted corneal sensation as a compensatory mechanism [42–44]. When diabetes persists for more than 20 y, the corneal sensitivity is so severely damaged by a long-term neuropathy that it cannot elicit blinks in response to the unfavorable conditions, and the blinking frequency hence fell down in these groups of diabetic patients.

In conclusion, our study demonstrates the importance of stratifying diabetic patients based on the disease duration, which has been ignored in the previous studies on ocular surface dysfunctions in the diabetics. In addition, our results show that there are significant differences in multiple ocular parameters among the subgroups with different diabetic durations, thereby indicating that long-term hyperglycemia and dysregulated metabolism may lead to exacerbating tear film abnormalities and ocular surface disorders.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The authors alone are responsible for the content and writing of the paper.

Conflicts of Interest

The authors report no conflicts of interest.

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