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Evaluation of the corneal endothelium in patients with diabetes mellitus type I and II

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Summary. Background: The aim of the present study is to determine corneal physiology and endothelial morphology after proper image analysis technique in type I and II diabetic patients. The HbA1c level and the grade of retinopathy were also recorded and correlated with the endothelial parameters. Methods: 41 eyes of 21 patients with type I and 59 eyes of 30 patients with type II diabetes mellitus (mean age was 40.97±15.46 and 64.36±10.47 years) were examined and compared to age-matched controls. Endothelial cell density (ECD), mean cell area, coefficient of variation of cell area, central corneal thickness, intraocular pressure, and grade of retinopathy were recorded. Results: There was a statistically significant decreased endothelial cell density in type I disease (2428±219 cell/mm^2) in comparison with healthy subjects (2495±191 cell/mm^2, P=0.02). The diabetic corneas were thicker than normal (P=0.001). The HbA1c level was inversely correlated with the ECD (r=-0.60; P<0.0001) and correlated with the mean endothelial cell area (r=0.60, P<0.0001). Significant correlation was observed between the endothelial morphology and grade of diabetic retinopathy (r=-0.40, ECD; r=0.38, mean cell area; P=0.01 for both). In type II diabetes mellitus no significant difference was found in the evaluated values. Conclusions: The present study disclosed the alteration of the corneal endothelial morphology in type I diabetes mellitus as compared to normal subjects. The results indicated that type I diabetic corneas are more susceptible to environmental changes than type II corneas.

Key words: Diabetes mellitus, Corneal endothelial morphology, HbA1c, Diabetic retinopathy

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Introduction

In diabetes mellitus there is an increased incidence of corneal complications. Clinical evidence proved that patients have functional abnormalities, such as recurrent corneal erosion, persistent epithelial defect, corneal oedema, and increased endothelial permeability after intraocular surgery (Perry et al., 1978; Schultz et al., 1981; Saini and Khandalavla, 1995; Sanchez-Thorin, 1998). Decreased corneal sensitivity and neutrophic corneal ulcers have also been reported (Schwartz, 1974; Hyndiuk et al., 1977).

There are several but still controversial reports on the corneal endothelium morphology in the disease. Earlier studies reported that the mean endothelial cell density was similar to normal subjects, with increased polymegethism, pleomorphism, in most cases with increased corneal thickness, and elevated intraocular pressure (IOP) (Pardos and Krachmer, 1980; Busted et al., 1981; Schultz et al., 1984). However, recent reports found altered endothelial morphology and function with decreased corneal endothelial cell density as compared to normal controls (Roszkowska et al., 1999; Inoue et al., 2002; Lee et al., 2006).

The results of the previous studies may derive either from different diabetic population (type I or II patients) or from different endothelial analysis methods, using contact and non-contact specular microscopes. Both techniques require the correction of cell count depending on magnification, corneal thickness, and the latter also on corneal curvature (Isager et al., 1999, 2000; Módis et al., 2002).

The aim of the present study is to determine central corneal thickness, intraocular pressure and endothelial morphology, such as cell density, cell area, and coefficient of variation of cell area after proper image analysis technique in type I and II diabetic patients in comparison with normal subjects. The general aspects of

the disease such as duration, serum glucose and glycosylated haemoglobin level (HbA1c), and the grade of retinopathy were also determined and correlated with the endothelial parameters.

Materials and methods

Patients and controls

Forty-one eyes of 21 patients (9 females, 12 males) with insulin dependent type I diabetes mellitus and 59 eyes of 30 patients with non-insulin dependent type II diabetes mellitus (20 females, 10 males) were recruited. The patients' characteristics are presented in a table (Table 1). For all patients in the type I group insulin therapy was initiated immediately after diagnosis. All procedures adhered to the tenets of the Helsinki Declaration, and the patients gave informed consent to their participation. Patients with previous ophthalmic disorder, contact lens usage, glaucoma and intraocular surgery were excluded from the study.

Both diabetic groups were compared with age-matched normal subjects. Control group I (served as a control for diabetes type I) included 40 eyes of 22 subjects (9 females, 13 males) with the mean age of 40.45±15.16 years. Control group II (served as a control for diabetes type II) consisted of 60 eyes of 30 subjects (15 females, 15 males) with the mean age of 62.69±13.38 years. Normal subjects were determined who had no previous or present ocular disease and had negative history of contact lens usage and intraocular surgery.

Patients underwent slit-lamp examination, IOP measurement, dilated fundus inspection and were submitted to specular microscopic investigation. To avoid diurnal fluctuation of corneal thickness, measurements were carried out after 2:00 pm as suggested earlier (du Toit et al., 2003).

Specular microscopy

Specular microscopy was performed with wide field contact specular microscope (Tomey EM-1000, Tokyo, Japan) in local anaesthesia by the same trained investigator (LM). The endothelium was focused sharply, and video frames were recorded directly onto a personal computer. Three to ten photographs were taken from the central corneal region to perform cell analysis (Fig. 1). The best 3 good quality specular images, scaled accurately with a 0.04 mm^2 grid including approximately 90 cells, were used for image analysis. Focus values indicating corneal thickness were read from the monitor and the data were stored on the computer (Seitz et al., 1997). Endothelial cell density and additional morphologic parameters, such as mean endothelial cell area and coefficient of variation of cell area were also determined with the image-analysis software (Tomey EM-1100, version 1.2.2.)

Table 1. Demographic and clinical characteristics of patients with type I and II diabetes mellitus.

	DM type I	DM type II
Age (years)	40.97±15.46	64.36±10.47
Duration of the DM (years)	10.88±8.06	13.61±6.50
Blood glucose (mmol/l)	10.81±4.94	11.17±3.15
HbA1c (%)	8.55±1.83	8.79±2.01
Stage of diabetic retinopathy	1.10±1.56	1.31±1.53

Correction of cell count

Calibration of the instrument was performed before the study. Conversion factors were introduced and used to ensure accurate cell count as provided by the manufacturer. The following equation was used to determine precise endothelial cell density:

$ECD\ (corr.) = ECC \times (F / 10.566)^2$

ECD corr. = corrected endothelial cell density
ECC = raw endothelial cell count
F = image focus (corneal thickness)
10.566 = conversion factor, provided by the manufacturer

Fundus examination

Dilated ophthalmoscopic investigation was performed in all patients by the retina specialist of our department (ÁK-B). After fundus photography, if necessary, patients were submitted to fluorescein angiography. The International Clinical Diabetic Retinopathy Disease Severity Scale was used to classify the stage of diabetic retinopathy (Wilkinson et al., 2003): 0, No apparent retinopathy; 1, Mild non-proliferative diabetic retinopathy; 2, Moderate non-proliferative diabetic retinopathy; 3, Severe non-proliferative diabetic retinopathy; 4, Proliferative diabetic retinopathy.

Statistical analysis

Descriptive statistical results were described with mean, standard deviation (SD), minimum and maximum values. Mann-Whitney U unpaired sample test was applied for comparison between groups. For bivariate correlation analysis, Spearman's rank correlation "r" was used. A P-value ≤0.05 was considered statistically significant. The patient age, duration of the disease, HbA1c, glucose level and intraocular pressure were recorded and used as independent variables. Correlation analysis was carried out between the fundus appearance and the endothelial morphologic parameters to determine the relationship between the presence or severity of diabetic retinopathy and diabetic keratopathy.

Results

Type I diabetes mellitus

The results of the corneal parameters are summarized in a table (Table 2). There was a statistically significant decreased cell density (P=0.024), increased mean endothelial cell area (P=0.001), coefficient of variation of cell area (P=0.002), and corneal thickness (P=0.001) in diabetic corneas as compared to the normal subjects (Fig. 2). No difference was present in the IOP values (P=0.25). The HbA1c level was inversely and significantly correlated with the endothelial cell density (r=-0.60; P<0.0001) and significantly correlated with the mean endothelial cell area (r=0.60, P<0.0001) (Figs. 3, 4). Statistically significant correlation was observed between the glucose level and the morphologic parameters (r=-0.35, P=0.023, endothelial cell density; r=0.36, P=0.022, endothelial cell area), pachymetry values (r=0.33, P=0.037). Statistical analysis revealed a significant negative correlation between the cell density and duration of the disease/insulin therapy (r=-0.38, P=0.014). Stage of diabetic retinopathy reflected by fundus appearance correlated significantly with endothelial cell density (r=-0.40, P=0.01) and mean cell area (r=0.38, P=0.015).

In type I diabetic group, endothelial cell density (r= -0.38, P=0.013) and mean cell area (r=0.41, P=0.008) correlated significantly with the patient age.

Type II diabetes mellitus

The evaluated parameters were also summarized in Table 2. No statistically significant difference was found in the endothelial morphology, corneal thickness, and IOP between diseased and normal eyes. In contrast to type I disease, no correlation was detected either between HbA1c or blood glucose level and endothelial

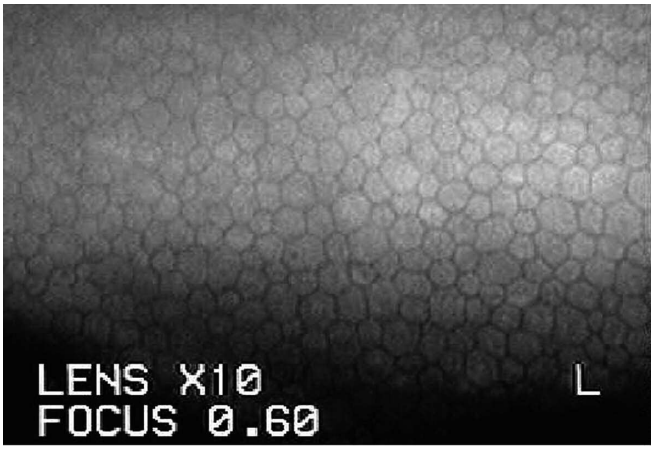

Fig. 1. Normal endothelial cell density and distribution in control group I. (Specular image, x 10).

Table 2. Descriptive parameters of type I and II diabetic corneas in comparison with their control groups.

	DM type I (n=41)	Control I (n=40)	$P_{DM\ I}$	DM type II (n=59)	Control II (n=60)	$P_{DM\ II}$
Cell density (cells/mm^2)	2428±219	2495±191	0.02*	2330±251	2354±186	0.56
Mean cell area (μm^2)	410.6±36	394.84±32	0.001*	426±46	420±30	0.64
Coefficient of variation	0.44±0.08	0.38±0.07	0.002*	0.44±0.08	0.44±0.07	0.61
Corneal thickness (μm)	570±40	550±40	0.001*	560±30	560±40	0.61
IOP (mmHg)	14.62±3.7	14.53±3.2	0.25	14.86±3.51	15.16±2.95	0.18

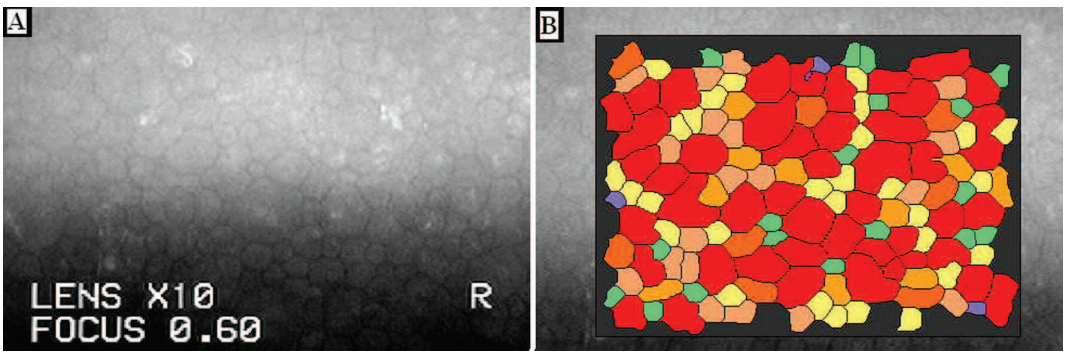

Fig. 2. Enlarged endothelial cells in patients with diabetes mellitus type I **(A)** and the same picture with image analysis technique **(B)**. Note that the pathologic baloon cells are in red. (Specular image, x 10).

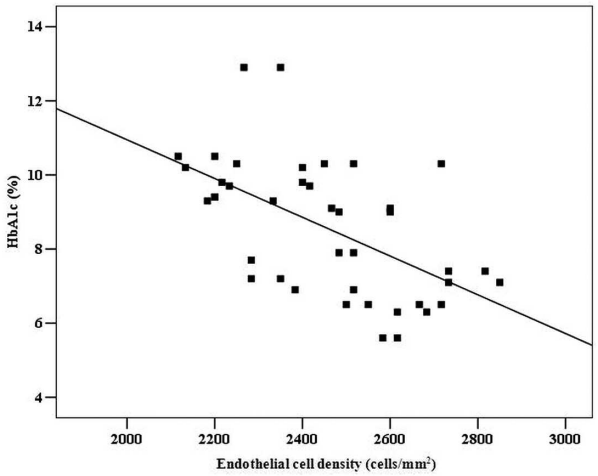

Fig. 3. The correlation of HbA1c level with the endothelial cell density in type I patient group (r=-0.60; P<0.0001; Spearman's rank correlation).

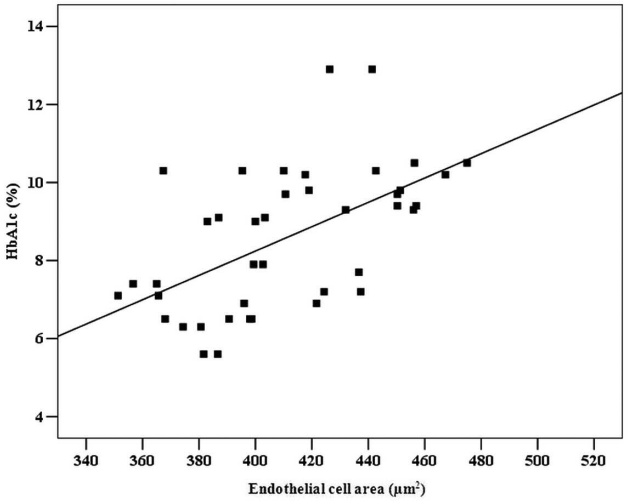

Fig. 4. The correlation of HbA1c level with the mean endothelial cell area in type I patient group (r=0.60; P<0.0001; Spearman's rank correlation).

Table 3. Summary of corneal morphologic and physiologic parameters available in the literature which compare diabetics to normal subjects.

	Specular microscopy	DM type	ECD	CV	CCT	IOP	Correlation
Pardos and Krachmer, 1980	contact	IDDM	=	na	na	na	na
Busted et al., 1981	noncontact	IDDM	=	na	↑	=	yes (ECD with duration of DM) no (CCT with blood sugar)
Schultz et al., 1984	specular camera	IDDM/NIDDM	=	↑	=	na	na
de la Messeliere and Renard, 1987	contact	IDDM/NIDDM	↓	na	ns	na	no (ECD with duration of DM)
Itoi et al., 1989	ns	NIDDM	na	↑	na	na	na
Matsuda et al., 1990	wide-field	NIDDM	=	↑	na	na	no (endothelial morphology with duration of DM and HbA1c)
Keoleian et al., 1992	wide-field	IDDM	=	↑	=	↑	no (endothelial morphology and function with duration of DM and HbA1c)
Frueh et al., 1995	confoscan	IDDM/NIDDM	=	na	na	na	na
Weston et al., 1995	wide-field contact	IDDM/NIDDM	=	=	↑	=	yes (endothelial morphology and severity of diabetes)
Larsson et al., 1996	wide-field contact	IDDM	=	↑	↑	na	yes (endothelial morphology with duration of DM) no (endothelial morphology with retinopathy)
		NIDDM	=	=	=	na	no (endothelial morphology with HbA1c)
McNamara et al., 1998	wide-field contact	IDDM	↓* =**	=* ↑**	↑	↑	yes (hyperglycemia affects corneal hydration control)
Roszkowska et al., 1999	wide-field contact	IDDM/NIDDM	↓	↑	↑	na	na
Siribunkum et al., 2001	contact	ns	↑	=	=	na	yes (CCT, pleomorphism, polymegathism with duration of DM no (corneal changes with glycemic control)
Inoue et al., 2002	noncontact	NIDDM	↓	↑	=	na	no (ECD with duration of DM, HbA1c, BUN, and creatine)
Quadrado et al., 2006	confoscan	NIDDM	=	na	na	na	no (disease state factor had no influence on relative cell density variation)
Lee et al., 2006	noncontact	IDDM	↓	↑	↑	na	no (endothelial morphology with duration of DM) yes (CCT with duration of DM)
Shenoy et al. 2009	confoscan	ns	↓	↑	na	na	yes (endothelial viability with grade of retinopathy)

DM: diabetes mellitus; ECD: endothelial cell density; CV: coefficient of variation of cell area; CCT: central corneal thickness; IOP: intraocular pressure; ↑: evaluated parameter higher in diabetes than in controls; ↓: evaluated parameter lower in diabetes than in controls; IDDM: insulin dependent diabetes mellitus; NIDDM: non-insulin dependent diabetes mellitus; na: not available; ns: not specified; *: central area; **: temporal area

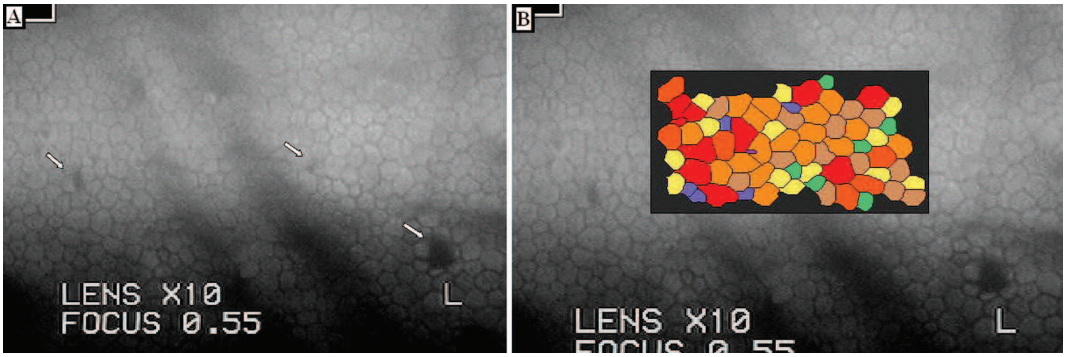

Fig. 5. Age related endothelial changes (corneal guttae, see arrows) in patients with diabetes mellitus type II **(A).** Some enlarged cells are also present **(B)**. Focus value indicates corneal thickness. (Specular image, original magnification x 10).

parameters. The duration of the disease did not correlate significantly with the corneal morphologic results (r=0.02, P=0.891, endothelial cell density; r=-0.01, P=0.932, corneal thickness). Spearman's test did not disclose significant correlation between the severity of diabetic retinopathy and endothelial cell parameters (r= -0.01, P=0.967, endothelial cell density; r=0.01, P=0.921, endothelial cell area).

For type II patients, none of the endothelial parameters was found to correlate with age (r=0.05, P=0.718, endothelial cell density; r=-0.05, P=0.713, endothelial cell area) (Fig. 5); however, central corneal thickness correlated negatively with patient age (r=-0.44, P<0.0001).

Discussion

The most conspicuous finding of the present study is the altered morphology of the corneal endothelium in patients with type I diabetes mellitus. The reduction of the mean cell density was associated with increased mean endothelial cell area and coefficient of variation in comparison with age-matched normal controls. These morphologic changes were accompanied with increased corneal thickness and normal intraocular pressure. However, these alterations could not be detected in type II diabetes mellitus.

The present observations are similar to those reported in the pathologic alterations of the corneal endothelium in diabetes mellitus (de la Messeliere and Renard, 1987; Itoi et al., 1989; McNamara et al., 1998; Roszkowska et al., 1999; Inoue et al., 2002; Lee et al., 2006). These previous studies also proved significantly increased mean endothelial cell area, coefficient of variation and decreased hexagonality in patients with diabetes mellitus type I and II. However, most of the earlier papers found no significant difference in the mean endothelial cell density between diseased and normal group of patients (Pardos and Krachmer, 1980; Busted et al., 1981; Schultz et al., 1984; Matsuda et al., 1990; Keoleian et al., 1992; Frueh et al., 1995; Weston et al., 1995; Larsson et al., 1996; Quadrado et al., 2006). In contrast, the recent studies available in the literature described significantly lower endothelial cell density in diabetic corneas in comparison with normal population (McNamara et al., 1998; Roszkowska et al., 1999; Inoue et al., 2002; Lee et al., 2006; Shenoy et al. 2009). Our findings were similar to these recent reports.

These different results on endothelial cell density may derive from the different specular and image analysis techniques, diabetic control, duration of the disease, and statistical methods used in different studies. The techniques and results on diabetic corneas are summarized in a table (Table 3).

It was described earlier that it is essential to record a minimum number of 3 good quality images, preferably by the same investigator (McCarey et al., 2008). More importantly, that for the evaluation of the accurate cell density, the correction of the cell count is essential to normalized magnification after proper calibration of the instrument (Isager et al., 1999, 2000; Módis et al., 2002). Specular image magnification is influenced by the corneal thickness. There is a linear correlation between such values, and an increase in corneal thickness results in an increase in cell count. An additional important factor for the proper image analysis is the number of analysed cells. It was stated previously that at least 75 cells are necessary for precise analysis (Doughty et al., 2000). The present investigation considered these suggestions during study design and implementation. Before the study the microscope was re-calibrated, the same investigator counted approximately 90 cells and the cell density was recorded after correction of thickness. In our opinion these factors are essential for proper image analysis technique.

Opposite to type I disease, the endothelial cell density and morphology was normal in type II diabetic patients in comparison with healthy age-matched subjects. It is known that endothelial cell density and hexagonality gradually decreases with age (Laing et al., 1976; Laule et al., 1978; Yee et al., 1985; Faragher et al., 1997; Bourne et al., 1997). These patients and their controls were from an older population, therefore these changes are similar and mimic age related alterations as described earlier (Larsson et al., 1996).

A further striking feature of this report was the inverse correlation between HbA1c and endothelial cell density in diabetes mellitus type I. This result suggests

that endothelial morphology may relate to hyperglycaemia, especially insulin deficiency. This serves as further evidence that type I diabetic corneas with poor diabetic control are more susceptible to intra-, and extraocular alterations, such as iatrogenic trauma (intraocular surgery) in the microenvironment. Our finding was in contrast to those previous investigations, which demonstrated no such relationship between endothelial morphology and glycosylated haemoglobin level (Matsuda et al., 1990; Keoleian et al., 1992; Larsson et al., 1996; Siribunkum et al., 2001; Inoue et al., 2002). However, some studies disclosed correlation between cell density and duration or severity of the disease, and even with the grade of retinopathy (Busted et al., 1981; Weston et al., 1995; Larsson et al., 1996; Saini and Mittal, 1996). In cases of type I diabetic patients the present study proved a significant correlation between the severity of diabetic retinopathy and keratopathy.

With the reduction of cell density we also detected significantly thicker corneas in the type I diabetic group. This was consistent with most previous studies evaluating corneal endothelial morphology and corneal thickness (Busted et al., 1981; Weston et al., 1995; Larsson et al., 1996; McNamara et al., 1998; Roszkowska et al., 1999; Lee et al., 2006; Su et al., 2008). The presumed mechanism is that reduced endothelial cell density causes reduced function, resulting in swelling of the corneal tissue. However, these changes in corneal structure and function are not clearly understood. The aldose reductase as the first enzyme of the polyol pathway is detected both in the epithelium and endothelium of the cornea by immunohistological studies (Akagi et al., 1984). This enzyme is responsible for the intracellular accumulation of polyols to extremely high levels, creating an osmotic imbalance leading to swelling and rupturing of cells, and may be responsible for the endothelial alterations in diabetic corneas (Kim et al., 1992; Ohguro et al., 1995).

Hyperglycaemia can also help the formation of advanced glycation end-products (AGEs), which alter protein structure and function, and participate in diabetic long-term complications. These heterogeneous molecules interact with their receptors (receptors for AGEs, RAGEs) found on many cell types, especially on those, which play a role in diabetes (Ahmed, 2005; Stitt and Curtis, 2005). This interaction leads to the production of free radicals, inflammatory molecules and has a considerable role in diabetic complications, such as retinopathy, cataract, atherosclerosis, neuropathy, and delayed wound healing (Ahmed, 2005; Stitt and Curtis, 2005).

In summary, the present study disclosed the alteration of the corneal endothelial morphology in type I diabetes mellitus compared to normal subjects after proper endothelial image analysis technique. The mean level of HbA1c demonstrated linear and significant correlation with the mean cell area, and inverse correlation with the cell density. Moreover, in type I patients a significant correlation was present between the severity of diabetic retinopathy and keratopathy. Therefore, glycaemic control is essential not only for the control of diabetic retinopathy, but also in the management of corneal complications. These changes indicate that type I diabetic corneas are more susceptible to environmental changes as compared to type II corneas.

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