Original Article

Corneal Endothelial Morphology in Type II Diabetic versus Non-Diabetic Individuals using Specular Microscopy

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ABSTRACT

Introduction: Diabetes is one of the major causes of comorbidities in elderly patients. Impaired vision and blindness in chronic diabetic population occurs due to poor corneal wound healing, corneal oedema, and other abnormalities of corneal layers as a result of accumulation of toxic products within cornea. Literature has shown corneal endothelial changes in diabetes in different populations but study on the Nepali population is sparse.

Objective: To compare the corneal endothelial parameters between type II diabetics and non-diabetics.

Methodology: A hospital-based, analytical, cross-sectional study was conducted after ethical approval from 2022 January to 2023 June using a CEM 530 NIDEK Spectral microscope to analyse the Endothelial Cell Density (ECD), Central Corneal Thickness (CCT), Hexagonality (HEX), and Coefficient of Variance (CoV). After meeting the selection criteria, 74 type II diabetic cases and 74 (age and sex-matched) controls were enrolled in this study using consecutive sampling method.

Result: The ECD was relatively higher in OD of diabetics than controls (2756.3 ± 228 cells/mm2 vs. 2748.7 ± 279.4 cells/mm2). The CoV was significantly higher in diabetics (OD 32.5 ± 5.4; OS 32 ± 4.7 vs. OD: 30.9 ± 3.8, OS: 30.5 ± 3.4). The HEX was significantly decreased in diabetics (OD: 65.5 ± 4.1, OS: 65.4 ± 5 vs. OD: 67 ± 5.2, OS: 67.1 ± 4.6). The CCT was significantly increased in diabetics (OD: 531.6 ± 29.9µm, OS: 538.3 ± 28.9µm vs. OD: 519.2 ± 32.3µm, OS: 526.1 ± 31.2µm). The duration of diabetes showed no significant difference in corneal parameters. However, diabetics with HbA1c <6.5 had significantly increased CCT than those with HbA1c >6.5.

Conclusion: There were significant changes in corneal endothelial morphology in patients with type II diabetes.

Key words: Corneal endothelium; diabetic mellitus; specular microscopy.

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INTRODUCTION

Corneal endothelium is the innermost layer of the cornea. It is a single layer of closely interdigitated hexagonal cells which are arranged in a mosaic pattern. The endothelial cells regenerate through enlargement and spread of residual cells or perhaps peripheral stem cells. These changes can be observed by specular microscopy as polymegathism (variability in cell size) and polymorphism (variability in cell shape) (Brar, 2020).

Specular microscopy is the study of the layers of cornea under very high magnification which is 100 times greater than slit-lamp biomicroscopy. It is mainly used to assess the endothelium. The corneal endothelium is evaluated in terms of cellular size, shape, density and distribution. It can be used to evaluate the functional reserve of the corneal endothelium before any intraocular surgery, evaluate the donor cornea in eye banks before keratoplasty, and study any underlying pathology in the corneal endothelium under greater magnifications in conditions such as Fuch's endothelial dystrophy, posterior polymorphous dystrophy etc (Salmon and Kanski, 2020). Specular microscopy has also been advocated preoperative analysis of cataract surgery to ensure better quality of postoperative vision (Yanoff and Duker, 2019).

Diabetes mellitus (DM) is one of the major comorbidities in elderly patients. Impaired vision and blindness occur due to poor corneal wound healing, corneal oedema, endothelial and epithelial basement membrane abnormalities as a result of toxic end product accumulation within the cornea of chronic diabetics (Shih et al., 2017). Research and studies around the globe have shown corneal endothelial changes in diabetes in different populations. However, no such studies have been done in our setting or hospital.

METHODOLOGY

This was a hospital-based, analytical cross-sectional study conducted at Nepal Eye Hospital, Tripureshwor, Kathmandu, after approval from Institutional Review Board of National Academy of Medical Sciences (Reference Number: 1481/2078/79) from 2022 January to 2023 June. Patients above 20 years of age, diagnosed as Type II Diabetics (Diabetic Group) along with age and sex-matched Non-diabetics (Non-diabetic Group) individuals were included in the study. Whereas patients with ocular trauma or infection, a history of intraocular surgeries, contact lens users, ocular disease (Glaucoma, Corneal dystrophies, Brown cataract, Retinal vascular diseases, Anterior uveitis, High refractive error), and patients with other metabolic diseases, connective tissue disorders and collagen vascular disease were excluded from the study. The sample size was determined with formula below:

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \, X \, (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

Here, n = sample size; $z_{\alpha/2}$ = 3.9 at 99.99% of confidence interval = 0.01%; Z_{β} = 2.33 at 99% of confidence interval = 1%; σ_1= standard deviation (SD) of mean ECD in type 2 DM individuals = 300.7; σ_2= SD of mean ECD in non-diabetic individuals = 221; μ_1= mean ECD in type 2 DM individuals = 2521.3; μ_2 = mean ECD in non-diabetic individuals = 2629.3 (Kadri et al., 2021).

Hence,

$$n = \frac{(3.9 + 2.33)^2 \text{ X } (300.7^2 + 221^2)}{(2521.3 - 2629.3)^2}$$

$$= \frac{6.23 \text{ X } 139261}{11664}$$

= 74.3 =74

So, 74 diabetic patients and 74 controls (age and sex matched) were enrolled in this study, a consecutive sampling technique was used and informed written consent was sought from all the enrolled participants.

The CEM 530 NIDEK Specular microscope was used for analysis of the key parameters which were endothelial cell density, central corneal thickness, hexagonality and coefficient of variance. The procedure was explained to the patient and then asked to sit comfortably on a chair, in front of the specular microscope (An auto-shot option was chosen by the fixed frame method). Then he/she was asked to fix his/her eyes inside the device till the instrument automatically takes a clean image of the corneal endothelium and measures the parameters. Averages of three consecutive readings focusing at the center of the cornea were taken for the analysis.

The software IBM SPSS version 24 (IBM Corp., Armonk, N.Y., USA), was used for the statistical analysis. The continuous variables were presented as mean ± SD. The categorical variables were presented as absolute numbers and percentage. Statistical comparisons were done using independent sample t-test and analysis of variance (ANOVA) test. The p-value of <0.05 was considered significant.

RESULT

The mean age of diabetic patients was 55.2 ± 10.2 (35-76) years, and of non-diabetic individuals, it was 54.5 ± 10.7 (31-80) years, and around 102 (68.9%) of the cases were above 50 years; there was no significant difference between the groups (p = 0.677). Males accounted for 70 (47.3%) cases in the study population, whereas 78 (52.7%) were females, and there was no significant difference between the groups (p = 1.000). The ECD was relatively higher in diabetics than controls (OD: 2756.3 ± 228 cells/mm^2; OS: 2773.6 ± 229.4 cells/mm^2 vs. OD: 2748.7 ± 279.4 cells/mm^2; OS: 2772.7 ± 292.4 cells/mm^2) (p = 0.858; 0.983). The CoV was significantly higher in diabetics (OD 32.5 ± 5.4; OS 32 ± 4.7 vs. OD: 30.9 ± 3.8, OS: 30.5 ± 3.4) (p = 0.042; 0.025). The HEX was significantly decreased in diabetics (OD: 65.5 ± 4.1, OS: 65.4 ± 5 vs. OD: 67 ± 5.2, OS: 67.1 ± 4.6) (p =0.048; 0.023). The CCT was significantly increased in diabetics (OD: 531.6 ± 29.9µm, OS: 538.3 ± 28.9µm vs. OD: 519.2 ± 32.3µm, OS: 526.1 ± 31.2µm) (p =0.017; 0.01). Patients with diabetes for less than 10 years accounted for 46 (62.2%) cases, 26 (35.1%) had it for 10-20 years, and 2 (2.7%) had diabetes for more than 20 years. The duration of diabetes showed no significant difference in corneal parameters (Table 2). Of the diabetic patients, 39 (52.7%) had a glycated haemoglobin (HbA1c) level of less than 6.5, while 35 (47.3%) had a level greater than 6.5. Diabetic patients with HbA1c <6.5 had significantly increased CCT than those with HbA1c >6.5 (Table 3).

Table 1: Corneal parameters in diabetics and non-diabetics.

Corneal Parameter		Diabetic Group	Non-Diabetic Group	p-value
Endothelial cell density (cells/mm²)	OD	2756.3± 228.0 (2092-3237)	2748.7± 279.4 (1791-3437)	0.858
	OS	2773.6± 229.4 (2261-3315)	2772.7± 292.4 (1719-3658)	0.983
Coefficient of variation	OD	32.5± 5.4 (22- 54)	30.9± 3.8 (22-39)	**0.042***
	OS	32.0± 4.7 (22-49)	30.5± 3.4 (23-40)	**0.025***
Hexagonality	OD	65.5± 4.1 (54- 74)	67.0± 5.2 (53- 81)	**0.048***
	OS	65.4± 5.0 (54- 76)	67.1± 4.6 (53- 75)	**0.023***
Central corneal thickness	OD	531.6± 29.9µm (468- 601)	519.2± 32.3µm (454- 604)	**0.017***
	OS	538.3± 28.9µm (482-615)	526.1± 31.2µm (459- 615)	**0.015***

Values expressed in Mean± SD (Minimum- Maximum); Analysis: Independent t-test.

Table 2: Corneal parameters in relation to duration of diabetes.

Parameters	<10 years n = 46	10-20 years n = 26	>20 years n = 2	p-value
OD Endothelial Cell Density (cells/mm²)	2753.4± 233.5 (3237- 2092)	2760.8± 205.1 (2446- 3202)	2763.0± 538.8 (2382- 3144)	0.991
OS Endothelial Cell Density (cells/mm²)	2757.2± 232.5 (2261-3202)	2785.3± 219.7 (2415- 3315)	2998.0± 295.6 (2789- 3207)	0.335
OD Coefficient of Variation	32.3± 5.4 (24-54)	32.9± 5.6 (22-50)	34± 0.0 (34)	0.850
OS Coefficient of Variation	32.3± 4.8 (22-49)	31.5± 4.6 (23-43)	31.5± 2.1 (30-33)	0.757
OD Hexagonality	65.9± 3.1 (59-73)	64.7± 5.4 (54-74)	68.0± 4.2 (65-71)	0.315
OS Hexagonality	64.8± 4.5 (54-74)	66.3± 5.9 (56-76)	63.5± 2.1 (62-65)	0.431
OD Central Corneal Thickness	530.8± 29.9µm (473-598)	530.9± 29.8µm (468-601)	557.5± 31.8µm (535-580)	0.467
OS Central Corneal Thickness	538.6± 30.6µm (484-615)	535.8± 26.2µm (482-602)	563.0± 26.9µm (544-582)	0.446

Values expressed in Mean± SD (Minimum- Maximum); Analysis: ANOVA test.

Table 3: Corneal parameters in relation to glycated haemoglobin level.

Parameters	HbA1C <6.5 n = 39	HbA1C >6.5 n = 35	p-value
OD Endothelial Cell Density (cells/mm²)	2772.8± 227.9 (2317- 3237)	2737.9± 230.1 (2092- 3202)	0.515
OS Endothelial Cell Density (cells/mm²)	2750.6± 211.2 (2377-3207)	2799.2± 248.8 (2261- 3315)	0.367
OD Coefficient of Variation	32.6± 4.8 (24-50)	32.5± 6.1 (22-54)	0.951
OS Coefficient of Variation	32.2± 4.4 (22-43)	31.8± 5.0 (23-49)	0.695
OD Hexagonality	66.4± 3.3 (56-74)	64.6± 4.7 (54-73)	0.056
OS Hexagonality	65.7± 4.5 (56-74)	65.0± 5.6 (54-76)	0.573
OD Central Corneal Thickness	541.7 ± 26.5µm (487-598)	520.3± 29.7µm (468-601)	**0.002***
OS Central Corneal Thickness	548.2± 28.5µm (484-615)	527.3± 25.6µm (482-602)	**0.001***

Values expressed in Mean± SD (Minimum- Maximum); Analysis: Independent t-test.

DISCUSSION

The exact pathogenesis behind the diabetic keratopathy remains unanswered; however, an article published by (Shih et al., 2017) mentioned that an increase in blood glucose levels resulted in the formation of advanced glycation end products. The accumulation of advanced glycation end products could affect different parts of the cornea resulting in three principal types of tissue dysfunction with physiological effects which are: (1) Defective wound healing in the corneal epithelium, (2) Abnormalities of sub-basal nerves and (3) Loss of corneal endothelial pump function (Shih et al., 2017).

Firstly, the rise in the blood glucose promotes IGFBP3 (Insulin like Growth Factor Binding Protein 3) release, which in turn competitively inhibits IGF-1 (insulin-like growth factor 1). Hyperglycemia suppresses TGFb3 (Transforming Growth Factor b3), CNTF (ciliary neurotrophic factor), and EGFR (epithelial growth factor receptor). As a consequence, there is reduction in epithelial cell proliferation on one hand and increase in apoptosis on the other hand that will both delay the epithelial wound healing (Shih et al., 2017).

Secondly, DM will cause neuronal damage. Due to prolonged hyperglycaemia, there is accumulation of advanced glycation end products which promotes inflammation and oxidative stress. NGF (nerve growth factor) and sphingolipids are key to neuronal health and myelin formation, but their production is inhibited in hyperglycaemic states (Shih et al., 2017).

Thirdly, prolonged hyperglycaemia will also result in endothelial cell loss and impairment in pump function. Apart from these processes, due to loss of epithelial barrier, crosslinking of stromal collagen and matrix, and loss of the endothelial pump there will be swelling of the corneal stroma (the main bulk of the cornea) (Shih et al., 2017). High blood sugar levels will promote non-enzymatic cross-linking via. glycation, when bond is formed between the sugar and amino group of protein, thereby resulting in an increased central corneal thickness (Doughty and Zaman, 2000; Krueger and Ramos-Esteban, 2007).

In the year 2022 A.D. among 1,41,643 patients, 780 patients came for screening or treatment of diabetic retinopathy at Nepal Eye Hospital. Most of the patients were between 40-69 years of age. The population of the study had slightly female preponderance (52.7%).

Among the diabetic patients, most of them had been diabetic for less than 10 years (62.2%). Whereas, 53% of diabetic patients had HbA1c level of more than 6.5. Due to the shorter duration of diabetes in most of the patients, the study could not have depicted the actual effect of chronic hyperglycaemia in an avascular tissue such as cornea. The study has shown that there is statistically significant increase in the mean CCT ($p = 0.017, 0.015$) and the CoV ($p = 0.042, 0.025$) but reduction in HEX ($p = 0.048, 0.023$) in the type 2 diabetic population compared to the non-diabetic population.

There was no significant difference noted in terms of mean ECD. The results were comparable with the meta-analysis by Zhang et al. (2021), which showed that diabetes could decrease the HEX but increase CoV, however it showed that the diabetes decreased the corneal ECD in contrary to our findings. The study by Lee et al. (2006) on the corneal morphological parameters in DM patients and age matched, healthy control subjects showed that the diabetic subjects had greater CCT, reduced ECD and HEX, and more CoV of the corneal endothelium than the control. Similarly, in the study by Singh et al. (2023) there was reduction in ECD of diabetic corneas (2493 ± 330 cells/mm^2) compared to controls which was statistically insignificant ($p > 0.05$), whereas there were significant results in terms of CoV ($p < 0.05$), and CCT ($p < 0.001$) but insignificant difference in terms of HEX % ($p = 0.718$) between diabetic and control group. Through the present study, the corneal morphological changes were also correlated with the duration of diabetes and the glycaemic control where the CCT correlated negatively with HbA1c levels but there was no correlation between endothelial parameters and duration of diabetes.

From above results, it is also possible that, in diabetic population there is increase in the formation of abnormal endothelial cells of different shape and sizes due to which the hexagonality of normal corneal endothelial cells is lost thereby increasing the coefficient of variance and resulting in increased corneal thickness. The mean corneal endothelial count alterations may not correlate with just one-time measurement of HbA1c levels. There can be an insult to the corneal endothelium during the course of the disease during the fluctuation of high blood sugar levels, which can result in permanent damage to the endothelial cells. This might have been the cause of the absence of correlation with the duration of the disease.

Talking about strength of this study, previous studies in Nepal have not addressed the problem of corneal endothelial damage due to type II DM using a specular microscope. So, this study compared the corneal endothelial morphology between age and sex matched type II diabetic and non-diabetic patients which was not done before. This study might aid in the better understanding of corneal endothelial morphology in the diabetic and non-diabetic patients. The data obtained from it can be useful for future studies of the diabetic cornea in relation to various surgeries such as phacoemulsification, small-incision cataract surgery (SICS), keratoplasty, etc.

The limitations of this study could be due to all the samples being collected from a single hospital so may not represent all diabetic patients. The study would have been better if we could analyse the corneal endothelium of diabetic population at defined intervals to get the real picture of fluctuating blood glucose on the cornea. The long-term effect of diabetes in the cornea could not be evaluated. The sample included less number of patients with uncontrolled diabetes and less number of patients with long duration of diabetes. So, it does not represent changes in endothelium in chronic and uncontrolled diabetes. The study was confined to only Type II DM, while the other different types of DM were not included.

CONCLUSION

There were significant changes in corneal endothelial morphology in patients with Type II Diabetes. Hence, during the routine screening of eyes in diabetics, not just the retina, the cornea should also be evaluated routinely for the changes of keratopathy.

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