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Research Article

Corneal Endothelial Changes in Patients of Type 2 Diabetes Mellitus Using Specular Microscopy

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Received: March 28, 2017; **Published:** April 26, 2017

Abstract

Objectives: To compare corneal endothelial cell changes namely, endothelial cell density (ECD), percentage polymegathism (CV) and pleomorphism (6A) along with central corneal thickness (CCT) in patients of type 2 diabetes mellitus with normal age and sex-matched subjects.

To correlate corneal endothelial changes with diabetic retinopathy status, total duration of disease and glycemic control (HbA1c) levels.

Study Design: A case-control study in a tertiary care hospital of south India.

Population: 270 eyes of patients of type 2 diabetes mellitus and 270 eyes of age and sex matched controls were included in the study.

Method: Demographic data such as age, gender and relevant diabetic history was obtained. A detailed evaluation of both cases and controls including complete anterior segment evaluation and posterior segment evaluation was done to grade the retinopathy. Non-contact specular microscopy was used to study the corneal endothelium.

Results: The mean endothelial cell density (ECD) was found to be significantly lower in patients with type 2 diabetes mellitus as compared to controls (p value = 0.0086) No statistically significant difference was observed in the coefficient of variation of cell size (CV), hexagonality (6A). Apparently normal, minimal increase was seen in central corneal thickness (CCT) between cases and controls. Though the endothelial cell density decreased with increase in severity of retinopathy, this was not found to be statistically significant.

Conclusion: Patients of type 2 diabetes mellitus have a lower endothelial cell count and higher rate of endothelial cell loss when compared to normal controls. The endothelial changes appear similar to changes caused due to aging process suggesting accelerated aging in diabetic cornea. The changes in corneal endothelium are proposed to be a part of the endothelium syndrome seen in patients of diabetes mellitus.

Keywords: *Diabetes; Cornea; Endothelial Syndrome; Specular Microscopy; Senescence*

Introduction

Diabetes mellitus is considered a major non-communicable disease worldwide. Based on projections made by International Diabetes Federation (IDF), 80 % of new cases are expected to occur in the developing world and in India diabetic population is expected to double by year 2030 [1,2].

Diabetic retinopathy remains the most commonly studied manifestation in eye [3]. However, functional abnormalities have been documented in cornea too like decreased endothelial cell density (ECD), increased corneal thickness and corneal keratopathy [4-9].

Corneal endothelial cell is responsible for maintaining the transparency of the cornea [5]. There is limited ability of mitosis in corneal endothelium and once damaged, remaining cells enlarge to cover up the lost area [10]. There will be an increase in variation of cell area called polymegathism or coefficient of variation (CV) and index of hexagonality (6A) or pleomporphism [11].

Central corneal thickness (CCT) can be used as a marker of endothelial health and can be used to monitor corneal edema. There is a postulated association between corneal thickness and severity of diabetic retinopathy [12-14].

Age can always be considered as a confounding factor in studying disease of corneal endothelium and endothelial cell density has been found to decrease with age [15,16].

Specular microscopes used today are based on design suggested by Maurice., et al [17]. The modern noncontact specular microscope to study conreal endothelium employ automated interfacing for obtaining image through a discrete focussing technology [5,18].

Considering the huge diabetic population in India and paucity of literature especially in India, a study was proposed to evaluate corneal endothelial changes using specular microscope in patients of type 2 diabetic mellitus.

Methodology

The study undertaken was a case control study and the period of study was from July 2013 to June 2015. A total of 270 eyes (cases) and 270 eyes (control) were included in the study.

Cases and controls were defined as patients of type 2 diabetes mellitus and age matched non-diabetic individuals respectively attending outpatient Department of Ophthalmology of JIPMER.

Any patient with presence of conditions known to affect endothelial count like pseudoexfoliation, previous history of laser photocoagulation, intraocular surgery, chemical injury, uveitis, glaucoma or affected with corneal pathology like keratoconus, dystrophies were excluded from the study. Patients of diabetes mellitus type 1 or any long-term contact lens users were also excluded.

Demographic data such as age, gender and relevant diabetic history was obtained. A detailed evaluation of both cases and controls including complete anterior and posterior segment evaluation was done to grade the retinopathy.

Non-contact specular microscope (Konan NonconRobo; Konan Inc, Japan) was then used to image the corneal endothelium. Using a fixed frame method of analysis and KSS software, 80 - 100 endothelial cells were photographed in centre of cornea. Further morphometric analysis and automated cell analysis was done to obtain mean corneal endothelial cell density (ECD, cells/mm^2), coefficient of variation of cell size (CV), percentage of hexagonality (6A), central corneal thickness (CCT).

All statistical tests and analyses were based on average of data from both eyes. Data was analysed using Statistical Package for the Social Sciences (SPSS). The corneal descriptors endothelial cell density, coefficient of variation, percentage of hexagonality and central corneal thickness were obtained and were expressed as mean with standard deviation (mean ± SD).

The data was analysed for normality distribution. The mean and proportions were compared using Student's t-test and Chi square test (x^2test) respectively. The Student's t-test was employed to compare corneal descriptors between the cases and control groups. Evaluation of correlation of corneal descriptors in type 2 diabetes mellitus with grade of diabetic retinopathy, duration of diabetes and HbA1c levels were done. Pearson's correlation coefficient (r) values were used to assess any association between corneal descriptors and HbA1c levels.

Analysis of variance test (ANOVA) was used to determine the differences of means among 3 or more groups i.e. to assess statistical significance between corneal descriptors and stages of diabetic retinopathy. A value of $p \leq 0.05$ was considered to be statistically significant.

Results

The demographic profile of cases and controls are described in Table 1. The mean age for cases of type 2 diabetes mellitus and controls were 56.06 ± 7.01 and 54.21 ± 9.02 years respectively (p = 0.0618). The case group had 79 (58.5%) males and 56 (41.5%) females while the control group had 66 (48.9%) males and 69 (51.1%) females.

Age group (Years)	Cases (%)	Controls (%)
≤ 49	21 (15.56)	37 (27.41)
50 - 59	73 (54.08)	59 (43.71)
60 - 69	35 (25.92)	32 (23.70)
≥ 70	6 (4.44)	7 (5.18)
	n = 135 (100%)	n = 135 (100%)

Table 1: *Table showing age distribution of cases and controls.*

The mean duration of type 2 diabetes mellitus was 7.08 ± 4.89 years (mean ± SD). The cases were further subdivided based on the status of retinopathy. The mean HbA1c level was 8.21 ± 0.97%.

The mean ECD in diabetic group was 2658.6 ± 255.3 cell/mm^2 while in the control group was 2735.9 ± 227.0 cell/mm^2 and the results were statistically significant (p value = 0.0086). However, comparison of coefficient of variation (CV), hexagonality (6A) and CCT in the two groups did not yield any statistically significant results (table 2).

	ECD(cell/mm^2)	CV(%)	6A(%)	CCT(µm)
Cases	2658.6 ± 255.3	40.89 ± 5.63	46.07 ± 5.31	541.37 ± 59.7
Controls	2735.9 ± 227.0	40.46 ± 5.29	45.57 ± 4.89	539.38 ± 47.3
p value	0.0086	0.3940	0.4220	0.7619

Table 2: *Comparison of mean value of endothelial cell parameters between diabetic patients and control across all age groups (expressed in mean ± SD).*

ECD: Endothelial Cell Density; CV: Coefficient of Variation; 6A: Hexagonality; CCT: Central Corneal Thickness

When corneal parameters were compared between proliferative diabetic retinopathy (PDR) patients and control group, statistically significant variation was seen in ECD. Between the two groups (p value = 0.025) while coefficient of variation, hexagonality and CCT were not found to significantly vary between the two groups (table 3).

	ECD(cell/mm^2)	CV	6A	CCT(µm)
Cases(PDR*)	2638.1 ± 265.2	41.85 ± 4.75	45.18 ± 5.59	548.78 ± 83.54
Controls	2735.9 ± 227.0	40.46 ± 5.29	45.57 ± 4.89	539.38 ± 47.30
p value	0.025	0.145	0.674	0.372

Table 3: *Comparison of mean value between proliferative diabetic retinopathy patients and control group expressed in (mean ± SD).*

**PDR = Proliferative Diabetic Retinopathy*

The cases were further stratified into different age groups and corneal descriptors were compared in the subgroups. The age group more than 70 years only had statistically significant p value < 0.05. The analysis involving different age group did not yield statistically significant results for coefficient of variation, hexagonality and central corneal thickness as shown in table 4 a-d.

Age group (Years)	Cases (ECD)	Controls (ECD)	p value
≤49	2719.19 ± 214.97	2800.19 ± 216.91	0.176
50-59	2641.60 ± 261.68	2716.26 ± 236.81	0.091
60-69	2689.87 ± 261.03	2690.92 ± 219.52	0.986
≥70	2471.67 ± 212.44	2768.21 ± 190.10	0.022
Total	2658.64 ± 255.32	2735.93 ± 227.00	0.0086

Table 4a: *Comparison of mean endothelial cell density between diabetic patients and control across different age groups (expressed in mean ± SD).*

Age group (Years)	Cases (CV)	Controls (CV)	p value
≤49	41.07 ± 4.70	40.46 ± 4.36	0.620
50-59	40.82 ± 5.19	40.13 ± 5.01	0.443
60-69	40.61 ± 4.96	41.11 ± 4.70	0.677
≥70	42.75 ± 4.67	40.29 ± 2.91	0.270
Total	40.89 ± 5.63	40.46 ± 5.29	0.394

Table 4b: *Comparison of coffecient of variation (CV) between diabetic patients and control across different age groups (expressed in mean ± SD).*

Age group (Years)	Cases (6A)	Controls (6A)	p value
≤49	45.88 ± 5.82	45.53 ± 5.49	0.818
50-59	46.53 ± 5.29	46.13 ± 4.63	0.643
60-69	45.49 ± 5.24	44.86 ± 5.10	0.622
≥70	44.42 ± 4.65	44.29 ± 2.34	0.949
Total	46.07 ± 5.31	45.57 ± 4.89	0.422

Table 4c: *Comparison of hexagonality (6A) between diabetic patients and control across different age groups (expressed in mean ± SD).*

Age group (Years)	Cases (CCT)	Controls (CCT)	p value
≤ 49	537.14 ± 59.24	536.28 ±38.63	0.947
50 - 59	543.68 ± 31.27	544.47 ± 50.62	0.912
60 - 69	538.41 ± 98.77	533.41 ± 54.87	0.801
≥ 70	545.33 ± 29.48	540.14 ± 13.35	0.682
Total	541.37 ± 59.7	539.38 ± 47.3	0.762

Table 4d: *Comparison of central corneal thickness (CCT) between diabetic patients and control across different age groups (expressed in mean ± SD).*

The comparison of endothelial cell parameters according to duration of disease did not yield statistically significant results. The variation in CCT across the two groups was 20.71 µm but was not found to be statistically significant. However, it was of clinical significance (table 5).

Duration of diabetes	ECD(cell/mm^2)	CV	6A	CCT
< 10 years	2654.71± 258.06	40.94 ± 5.15	45.94 ± 5.40	536.00 ± 47.84
≥ 10 years	2669.84 ± 250.88	40.74 ± 4.62	46.43 ± 5.13	556.71 ± 84.04
p value	0.764	0.842	0.641	0.077

Table 5: *Variation of corneal endothelial parameters in type 2 diabetic patients according to duration of diabetes.*

The variation of endothelial cell density (ECD) with different stages of diabetic retinopathy was assessed. Though there was difference of 52 cell/mm^2 between no retinopathy and PDR groups, statistical significance was not noted. Other parameters (CV, 6A, CCT) were not found to vary significantly with different stages of diabetic retinopathy (table 6).

Diabetic Retinopathy	ECD(cell/mm^2)	CV	6A	CCT(µm)
No retinopathy (30)	2690.7 ± 243.2	39.86 ± 6.23	46.75 ± 5.88	542.60 ± 25.14
NPDR* (35)	2695.3 ± 262.2	41.17 ± 4.30	46.09 ± 4.26	525.38 ± 65.22
NPDR**(32)	2612.8 ± 248.9	40.39 ± 4.69	46.45 ± 5.55	548.90 ± 37.54
PDR***(38)	2638.1 ± 265.2	41.85 ± 4.75	45.18 ± 5.59	548.78 ± 83.54
Total (n=135)	2658.6 ± 255.3	40.89 ± 4.99	46.07 ± 5.31	541.37 ± 59.71
p value	0.485	0.379	0.638	0.308

Table 6: *Comparison of corneal endothelial parameters with severity of retinopathy in patients of type 2 diabetes.*

NPDR : Non proliferative diabetic retinopathy (mild to moderate)*

*NPDR**: Non proliferative diabetic retinopathy (severe to very severe)*

*PDR*** : Proliferative diabetic retinopathy*

The mean HbA1c level in patients of type 2 diabetes mellitus was 8.21 ± 0.97% (mean ± SD) in this study. The variation of endothelial cell parameters with HbA1c levels was not statistically significant.

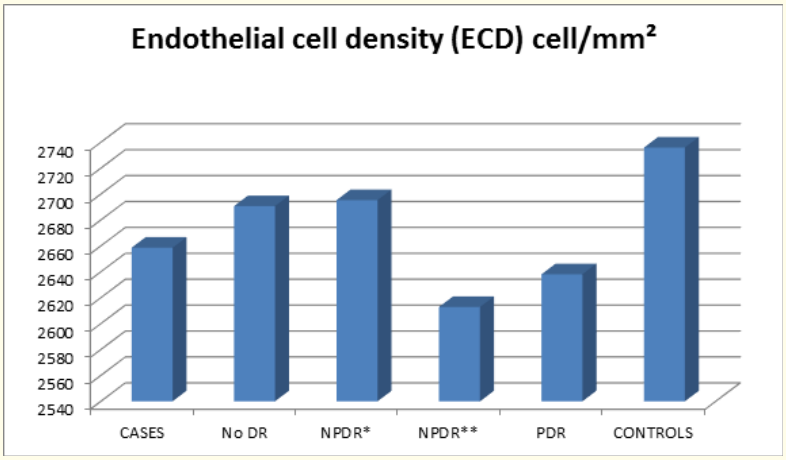

Figure 1: *Comparison of mean value of endothelial cell density between diabetic patients and control across all age groups expressed in cells/mm^2.*

NPDR: Nonproliferative diabetic retinopathy (mild to moderate)*

*NPDR**: Nonproliferative diabetic retinopathy (severe to very severe)*

Discussion

The defined objectives of this study were to analyze the effect of type 2 diabetes mellitus on endothelial morphology and functionality. In this study, age difference between the cases and controls was not statistically significant. (p value = 0.06). So, the groups were comparable.

The average duration of type 2 diabetes mellitus was 7.08 ± 4.89 years. However, this number may not be reflective of the actual duration of diabetes since type 2 diabetes remains asymptomatic at its onset [3].

The mean ECD was found to be significantly lower in patients with type 2 diabetes mellitus when compared with controls. (p value = 0.0086). The difference noted between the two groups was 2.8 %. Studies by Itoi., *et al*. and Matsuda., *et al*. involving less sample size (70 or less) did not report any statistically significant difference with respect to endothelial cell density [20-21].

Cell loss per year was higher in type 2 diabetics (0.45%/year) as compared to controls (0.22%/year). This may suggest higher corneal endothelial damage in diabetic patients even after excluding the effect of age. Inoue., *et al*. suggested in their study that the damage to endothelial cells caused by diabetes mellitus may be severe enough to nullify the effect of age [7].

This study assessed coefficient of variation and hexagonality in both the groups and found that it was not statistically significant. Previous studies by Inoue., *et al*. and Sudhir., *et al*. also did not find any significant variation [7,9].

There was a minimal increase in corneal thickness in diabetic patients suggesting that the effect of type 2 diabetes mellitus on the corneal thickness may be minimal. This findings is similar to one noted by Inoue., *et al* [7]. CCT did not increase significantly with ageing in both the groups.

The difference in endothelial cell parameters between two diabetic groups (less than 10 years and ≥ 10 years) were analysed. It was observed that there was not a statistically significant difference between the two groups. However, concluding that disease duration does not have a bearing on endothelial cell density may be difficult because duration of disease was based on history itself and many patients would have been undiagnosed diabetics for many years. Inoue., *et al*. and Lee., *et al.* have used similar criteria in their cross-sectional studies [7,19].

Comparison of PDR with control group yielded significant results in ECD. (p value = 0.025). HbA1c levels were not correlated to corneal endothelial parameters suggesting that these parameters may not be related to short term glycemic control.

Strength and Limitation

The study has been carried out in a fairly large sample size of 270 (135 cases and 135 controls). Previous studies had relatively lesser sample size [6,20,21]. It gives the normative values of endothelial cell parameters for healthy Indian population.

The duration of diabetes was not exact (study being a cross-sectional study) and was based purely on history itself. The actual duration may be different as diabetes mellitus in initial phase remains asymptomatic.

Similarly, HbA1c levels are based on glycemic control over a period of 3 months and thus corneal endothelial cell findings may not be correlated to HbA1c at one point of time. True assessment may be possible by conducting a longitudinal study. The total numbers of patients in late stages of diabetic retinopathy (though were higher compared to previous studies 6-8) appeared less to identify any significant difference.

Conclusions

Patients of type 2 diabetes mellitus have a lower endothelial cell count when compared to non-diabetic control group (p value = 0.0086). The rate of endothelial cell loss per year was found to be higher in diabetic group. (0.45% in patients of type 2 diabetes mellitus as compared to 0.22% in normal controls).

The duration of diabetes, severity of retinopathy (non-proliferative retinopathy and proliferative retinopathy) were not found to affect endothelial parameters to a greater extent to cause a statistically significant variation. Similarly, HbA1c levels were not correlated to any of the corneal endothelial cell parameters. The possible explanations for these findings could be that since type 2 diabetes mellitus is a chronic disease, it seems likely that subtle changes develop over a long period of time. So, at the time of examination, few systemic or ocular factors may not show any association.

The corneal endothelial changes seen in diabetes mellitus appear similar to changes caused due to aging process with a higher rate of endothelial cell loss. The diabetic endothelium being under stress has a higher propensity to decompensate. Specular microscopy can thus be used as a tool for prognostication of corneas of diabetic patients and more importantly it is imperative that corneal specular microscopy be done routinely preoperatively for diabetic patient even if on clinical examination cornea appears healthy. Whether the corneal endothelial changes in diabetes can be used to predict vascular endothelial changes leading to diabetic nephropathy, retinopathy and heart disease is yet to be elucidated. It could be considered as a subject of further research.

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Volume 6 Issue 4 April 2017