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Original Research Article

Corneal thickness and endothelial cell density in diabetic and non-diabetic patients: a hospital based comparative study

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ABSTRACT

Background: Diabetes mellitus is associated with structural changes in corneal endothelial cells and their thickness. The present study was done to compare the endothelial cell density (ECD), central corneal thickness (CCT) and morphology in diabetic and non-diabetic patients.
Methods: A cross-sectional study was conducted at Minto Ophthalmic hospital, BMC and RI Bangalore for a period of 20 months (October 2013 - May 2015). A total of 200 study subjects, 100 diabetics and 100 non-diabetic age matched controls were selected, and complete timed ophthalmic evaluation was performed. Specular microscopy was performed on all patients for endothelial cell count assessment and corneal thickness was measured by Pachymeter. The data was analyzed and represented using descriptive statistics. 't' test was used for comparing the two groups.
Results: The mean endothelial cell density in diabetic group was significantly lower (2438.73±250.23cells/mm^2) compared to non-diabetic group (2599.88±168.16cells/mm^2) ($p<0.0001$). The mean Central corneal thickness in diabetic group was significantly higher (518.40±28.13 µm) compared to control group (490.14±24.31 µm) ($p<0.001$). The Co-efficient of variation percentage of the diabetics was higher than the non-diabetics but this difference was not statistically significant ($P>0.05$). The hexagonality percentage was significantly lower in diabetic group compared to the controls suggesting less pleomorphism in the diabetic group.
Conclusions: The study concludes that the endothelial cell density was lower and central corneal thickness was higher in diabetic patients compared with the non-diabetics. The altered endothelial morphology was significantly seen in the form of pleomorphism (hexagonality) but polymegathism was not significantly altered.

Keywords: Corneal thickness, Co-efficient of variation, Endothelial cell density, Hexagonality

INTRODUCTION

The term diabetes mellitus (DM) is described as metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs.[1] During the year 2014, the number of cases of diabetes worldwide is estimated to be around 422 million, of these more than 90% are type 2 diabetes. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014.[2] According to the recent estimate done by the International Diabetes Federation (IDF), South East- Asia (SEA) Region consisting of India, Sri Lanka, Bangladesh, Bhutan, Mauritius and Maldives, is home to more than 72 million adults with

diabetes in 2013 and is expected to exceed 123 million in 2035.[3] During the year 2012 in India, the proportional mortality due to diabetes was about 2 percent.[4]

While the most prominent manifestation of impaired macrovascular function in DM is accelerated atherosclerosis, microvascular dysfunction leads to nephropathy and retinopathy. Among the microvascular complications of diabetes, diabetic retinopathy (DR) is the most common and is the leading cause of blindness. Although the most common, DR is not the only ocular complication of diabetes; others include corneal dysfunction, cataract, glaucoma, neuropathy, ischemic optic neuropathy, and diabetic macular edema.[5,6]

Corneal diseases being one of the complications of DM, are difficult to manage like diabetic retinopathy. Functional abnormalities may induce increased corneal autofluorescence as measured by fluorophotometry as well as increased corneal endothelial permeability, although some researchers have reported that corneal endothelial permeability is not increased.[7]

Diabetes leads to increased aqueous humor glucose levels and inhibit directly the corneal endothelial function. Cornea significantly swelled less in hyperglycaemic state. The recovery rate is slower in diabetics even in euglycemic state. On studying the differences in the base line corneal structure, the diabetic patients showed less corneal swelling and reduced corneal recovery from hypoxia when compared to the normal population.[8]

The endothelial cell morphologic features are related to severity of Diabetes. The Diabetic corneas were thicker and more autofluorescent than the non –diabetic corneas. Diabetes mellitus affects the corneal hydration.[9] The endothelial morphology is influenced by many factors. The variants of endothelial morphology i.e., endothelial cell density (ECD), Co-efficient of variation (CV) and percentage of hexagonal cells are affected by age, race, and refractive errors.[10] Morphological abnormalities may induce a high coefficient of variation of cell area and a decrease in the percentage of hexagonal cells in the corneas of diabetic patients compared with those of non-diabetic patients.[7]

Regarding endothelial cell density in diabetic patients, one study has reported it to be decreased while others have reported that it is similar to values in non-diabetic patients.[11-14] Functional and morphological abnormalities in diabetic endothelial cells had not yet been defined. Hence the current study was undertaken with the objectives to compare the endothelial cell density and central corneal thickness (CCT) among diabetic and non-diabetic subjects and to compare the variants of endothelial morphology i.e., Co-efficient of variation and percentage of hexagonal cells among diabetic and non-diabetic subjects.

METHODS

A cross-sectional comparative study was conducted over a period of 20 months in the department of ophthalmology, Minto Ophthalmic Hospital, BMC and RI, Bangalore, India from October 2013 to May 2015. 100 diabetic patients (type 1 or type 2) who were already diagnosed and on treatment and 100 age matched healthy patients posted for cataract surgery between the age group 40 to 80+ years during the study period were selected by convenience sampling. Patients with history of previous ocular surgery, ocular trauma, primary or secondary glaucoma, corneal diseases and dry eye syndrome were excluded from the study. None of the patients used topical medications. 182 eyes of diabetic and 190 eyes of control healthy patients falling under the inclusion criteria were analysed.

The study was approved by the institute research and ethical committee and written informed consent was obtained from each patient after explanation of the nature of the study. All these selected patients were subjected to comprehensive ophthalmic examination including complete medical history, slit lamp examination, binocular indirect ophthalmoscopic fundus examination and intraocular pressure was recorded. Gonioscopy was performed whenever required.

Corneal thickness measurement

The corneal thickness was measured using ultrasonic pachymeter (quantel hand held pachymeter). Patients were instructed to look straight ahead at a fixation target located at 3 m. After having pushed the button to initiate corneal thickness measurements, the probe tip was gently positioned to touch the patient's cornea at its centre. The Pachymeter probe had to be perpendicular to the apex of the cornea. If the measurement was valid, a value appeared on the digital display. The mean value of three consecutive measurements was used for the statistical analysis. All measurements were taken by the same physician. An average of three measurements was taken for each eye.[15]

Endothelial cell count assessment

Non-contact specular microscopy was performed with the Tomey EM – 3000. The subject was positioned on the chin and forehead rest and asked to fixate on the red target. On proper alignment on the centre of the cornea, a bright central specular image of the central corneal epithelium was obtained. If the endothelial image displayed on the monitor was not in focus, the process was repeated. Up to 300 cells per image were counted in fixed areas of 0.135 mm (0.25 30.54mm). A proprietary automated cell counter recognition algorithm based on contrast differences and area based counting technique was used to assess the central endothelial density (ECD i.e., cells per square millimetre), variation in the size of the endothelial cells (CV-coefficient of variation) and

percentage of hexagonal cells. The mean cell area and the CV in the cell area (standard deviation divided by the mean cell area) were used as an index of the extent of the variation in cell area (polymegathism). The percentage of hexagonal cells in the area analyzed was used as an index of variation in the cell shape (pleomorphism).[16,17] All the results were recorded and tabulated for statistical analysis.

Statistical analysis

All the observations were recorded and tabulated in Microsoft excel, analysed using SPSS software version 18. The data was analysed and represented using percentage, proportions. mean±Sd for representing quantitative data. Independent samples 't' test was used for comparing the two groups.

RESULTS

In the present study, out of 200 study subjects, 100 subjects were diabetics and 100 subjects were non-diabetics each. 182 eyes of diabetic and 190 eyes of control healthy non-diabetic subjects falling under the inclusion criteria were included for analysis.

Table 1: Demographics of the diabetic and non-diabetic subjects.

Demographics	Diabetics (n=100)	Non-diabetics (n=100)	P value
Age in years (Mean±SD)	63.05±8.90	61.21±7.73	>0.05
Gender (n)			
Male	45	55	>0.05
Female	53	47	

*P<0.05 considered statistically significant

Among the diabetic patients 55.0% were females and 45.0% were males. The mean age was 63.05±8.90 years. The mean age of males was 65.15±9.20 years and of females 61.32±8.34years. Among the non-diabetic patients 53.0% were males and 47.0% were females. The mean age was 61.21±7.73 years. The mean age of males and females were 60.79±7.35 years and 61.5±8.17years respectively. There was no statistically significant difference between the mean age and sex distribution between the two groups (P>0.05) (Table 1).

Table 2: Age distributions in diabetic and non-diabetic subjects.

Age (years)	Diabetic (n=100)	Non-diabetic (n=100)
≤50	12 (12.0)	10 (10.0)
51-60	23 (23.0)	38 (38.0)
61-70	44 (44.0)	47 (47.0)
>70	21 (21.0)	05 (05.0)
Total	100 (100.0)	100 (100.0)

Figures in parenthesis indicates percentage

Majority i.e., 44.0% and 47.0% of the study subjects were in the age group of 61-70 years, followed by 23.0% and 38.0% in the age group of 51-60 years in diabetic and non-diabetic group respectively (Table 2).

Table 3 compares the outcome measures endothelial cell density (ECD) and central corneal thickness (CCT), age wise, between diabetics and non-diabetics. The mean corneal endothelial cell density was lower (2438.73±250.23 cells/mm^2) in diabetics than in non-diabetics (2599.88±168.16cells/mm^2) (P<0.05). The similar decrease in the cell density is seen in all the age groups and this difference was statistically significant. (P<0.05) The central corneal thickness was significantly higher (518.40±28.13 µm) in the diabetic group compared to the control group (490.14±24.31 µm) (P<0.05).

Table 3: Comparison of endothelial cell density and central corneal thickness of diabetics with age matched controls.

Variable	Age group	Diabetics (n=182)	Non-diabetics (n=190)	t value (95% CI)	P value
Endothelial cell density (cells/mm^2)	All ages	2438.73±250.23	2599.88±168.67	- 7.308 (-117.75 to -204.44)	<0.0001*
	≤50	2617.75±165.70	2809.10±91.27	- 13.875 (-164.27 to -218.52)	<0.0001*
	51-60	2460.10±142.43	2589.90±138.63	- 8.907 (-101.14 to -158.45)	<0.0001*
	61-70	2412.90±269.21	2581.87±173.62	- 7.221 (-122.90 to -214.89)	<0.0001*
	>70	2392.23±274.90	2439.60±101.85	- 2.222 (-5.469 to -89.330)	0.026*
Central corneal thickness	All ages	518.40±28.13	490.14±24.31	10.378 (33.613 to 22.906)	<0.0001*
	≤50	526.58±31.71	491.80±21.70	12.390 (40.299 to 29.260)	<0.0001*
	51-60	520.70±19.34	487.78±21.59	15.467 (37.105 to 28.734)	<0.0001*
	61-70	514.36±32.33	491.55±27.87	7.298 (28.956 to 16.664)	<0.0001*
	>70	524.61±23.09	486.40±18.88	17.505 (42.502 to 33.917)	<0.0001*

*P<0.05 considered statistically significant

Table 4 compares the endothelial cell morphology in the form of co-efficient of variation and hexagonality, age wise, between diabetics and non-diabetics. There was no significant difference in the co-efficient of variation for overall all ages (*P*>0.05) though the diabetic eyes have marginally higher polymegathism (38.62±4.24%) than the non-diabetic eyes (38.05±4.12%) except in the age group of > 70years where it is slightly lower in diabetic eyes. The hexagonality percentage were significantly lower (40.43±5.13%) in the diabetic group compared to the non-diabetic group (43.58±4.19%), similar differences observed in all the age group (P<0.05).

Table 4: Comparison of the endothelial cell morphology of diabetics with age matched controls.

Variable	Age group	Diabetics (n=182)	Non-diabetics (n=190)	T value (95% CI)	P value
Co-efficient of variation (%)	All ages	38.62±4.24	38.05±4.12	1.315 (1.422 to -0.282)	0.189
	≤50	37.16±5.06	35.80±4.96	2.617 (2.381 to 0.338)	0.009*
	51-60	38.60 ± 3.76	37.89±4.28	1.696 (1.532 to -0.112)	0.09
	61-70	39.47 ± 3.83	38.62±3.81	2.145 (1.629 to 0.070)	0.03*
	>70	36.66±3.49	38.0±2.34	-4.366 (-0.736 to -1.943)	<0.0001*
Hexago Nality (%)	All ages	40.43±5.13	43.58±4.19	- 6.498 (-2.196 to -4.103)	<0.0001*
	≤50	43.16±7.34	45.30±4.08	- 3.494 (-0.935 to -3.344)	<0.0001*
	5- 60	40.50±5.13	43.51±3.87	- 6.405 (-2.086 to -3.934)	<0.0001*
	61-70	40.25±5.13	43.57±4.26	- 6.802 (-2.360 to -4.279)	<0.0001*
	>70	39.47±3.70	40.20± 3.83	- 1.868 (0.038 to -1.498)	0.062

DISCUSSION

The endothelium of cornea is under metabolic stress in diabetic state.[18] Diabetic cornea may give the impression that is healthy, but actually it may suffer from many morphological features affecting its function later on.[19]

In the current study the mean age of the study subjects was 63.05±8.90 years and 61.21±7.73 years among diabetics and non-diabetics respectively which is comparable to study findings of Sahu PK et al showing a mean age of 63.38±7.31 years in diabetics and 64.00±8.32 years in non-diabetics.[20]

Morphological features of the corneal endothelium in diabetic patients recognized by a number of studies include a decrease in endothelial cell density (ECD) and pleomorphism (decrease in the percentage of hexagonal cells [the normal percentage >50%]) as well as polymegathism, which means increased coefficient of variation (CV) of cell area (CV values measured between 0.22 and 0.31 are considered normal and above 0.4 are abnormal) and increased central corneal thickness (CCT) which is similar to the current study findings which showed decrease in the endothelial density and increase in the central corneal thickness in the diabetic group compared to the non – diabetic group, the CV percentage of the diabetics was marginally higher than the non-diabetics in all age groups except for >70 years age and decreased percentage of hexagonality in the diabetic group compared to the controls suggesting more pleomorphism in the non-diabetic group.[21,22]

Kukadia G et al in his comparative study also noted that decreased endothelial cell density and increased corneal thickness in diabetes compared to the non-diabetic control group.[23] Paulsen AS et al also noted lower endothelial density and increase in corneal thickness in diabetic group.[24] Roszkowska AM et al studied on corneal endothelium evaluation in type I and type II diabetes found that all evaluated parameters were significantly different in both type I and type II diabetic groups, with reduction in the mean cell density of 5% in type II and 11% in type I diabetes in comparison to normal age matched normal controls. The central corneal thickness was significantly higher in diabetics.[25] Urban B et al in his study on corneal endothelium in children and adolescents with type I diabetes mellitus concluded that the lower corneal endothelial cell density and thicker cornea in children and adolescents with type I diabetes. Duration of diabetes is the factor that affects the ECD and CCT and this count predisposes to the corneal dysfunction.[26]

Lee JS et al studied the correlation of the endothelial morphology and the corneal thickness to the duration of the diabetic found that the endothelial morphological change in the diabetics and central corneal thickness increased in the diabetic compared to the non – diabetic population and correlated to the duration of diabetic.[27] Choo MM et al in the study of the corneal changes in type II diabetes mellitus in Malaysia found the type II diabetes causes a significant alteration in the state of the cornea including the reduction in the endothelial density and increased polymorphism and polymegathism but corneal thickness not affected.[21] Sudhir RR et al found that the cell density decreased with no difference in the Hexagonality, CV of the cell surface, and no difference in the corneal thickness.[22] Sahu PK et al also observed that

percentage hexagonality was higher in nondiabetes controls but was statistically not significant.

Galgauskas S et al in his study about age-related changes in corneal thickness and endothelial characteristics concluded that ECD and CCT decreased with age whereas CV and hexagonality were not dependent on age which can be related to present study to note the change observed in ECD, CCD, CV, hexagonality.[28]

Limitation of the study was that this study is a hospital based cross-sectional study, hence there a need for conducting a follow up study to know the corneal changes with increasing duration of diabetes and to have a representative sample, the study needs to be conducted in a larger sample and in a community-based setting to generalize the obtained results.

CONCLUSION

The endothelial cell density was significantly decreased, and central corneal thickness was significantly increased in diabetics compared to the non-diabetic group. The altered endothelial morphology was seen in the form of significant lower percentage of hexagonality (pleomorphism) but co-efficient of variation (polymegathism) was not significantly altered among diabetics and non-diabetics.

Recommendations

Diabetes is a chronic metabolic disease and it is common to have some association between the systemic and ocular factors influencing the corneal endothelium. Precautionary measures have to be taken in diabetics before any intra-ocular-procedures, prolonged period of contact lens wear, in glaucoma and use of drugs that affect the endothelium.

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