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Research Article

Window to Corneal Endothelium Health in Diabetics: Analysis with Specular Microscopy

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Abstract

Background: Diabetes mellitus has been postulated to alter the morphology of the cornea. The cornea consists of 5 layers. The Endothelium being the most important layer, consists of a monolayer of polygonal cells and that maintains corneal deturgescence throughout life by pumping excess fluid out of the stroma.

Aim of the Study: To compare the morphological characteristics of corneal endothelial cells in type 2 diabetic patients with age-matched healthy subjects in terms of the general characteristics, gender, and laboratory data of diabetic patients, including disease duration, HbA1c, type of medication.

Materials and Methods: A case-control study in tertiary health care centre, with a total of 50 diabetic patients and 50 healthy subjects were enrolled. Selected participants who qualified the inclusion criteria and were enrolled for the study after taking a written informed consent underwent complete ophthalmological examination. Corneal endothelial morphology and central corneal thickness were measured using a noncontact specular microscopy and compared 50 diabetic patients with 50 healthy controls. Laboratory data including serum fasting glucose, HbA1c levels were also recorded. Statistical analysis was undertaken with $P < 0.05$ significance.

Results: In this study, we identified significantly decreased Endothelial Cell Density and percentage of hexagonal cells, elevated average cell size and coefficient of variation in diabetic patients compared with the same parameters in healthy controls ($p = 0.000$). When correlation analysis was performed between corneal morphological features and laboratory data of diabetic patients, Endothelial Cell Density showed a significant negative correlation with diabetes duration.

Conclusion: As compared with healthy controls, Diabetic patients have definite more endothelial cell loss. Endothelial keratopathy is an important complication of type 2 diabetes. In that respect, we can suggest that endothelial keratopathy and endothelial cells should be evaluated more cautiously in diabetic patients.

Keywords: *Specular Microscopy; Corneal Endothelial Cells; Diabetes Mellitus*

Abbreviations

IDF: International Diabetes Federation; ADA: American Diabetes Association; RBG: Random Blood Glucose; DM: Diabetes Mellitus; DR: Diabetic Retinopathy; ECD: Endothelial Cell Density; CV: Coefficient of Variation; Percentage of Hexagonal Cells (6A); CCT: Central Corneal Thickness

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. DM is associated with damage and dysfunction of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [9].

Corneal endothelial cell is responsible for maintaining the transparency of the cornea [17,22]. There is limited ability of mitosis in corneal endothelium and once damaged, remaining cells enlarge to cover up the lost area. There will be an increase in variation of cell area called polymegathism or coefficient of variability (CV) and index of hexagonality (6A) or pleomorphism.

Central corneal thickness (CCT) can be used as a marker of endothelial health and can be used to monitor corneal edema. There is an association between corneal thickness and severity of diabetic retinopathy [3,4,12]. Age can always be considered as a confounding factor in studying disease of corneal endothelium as endothelial cell density has been found to decrease with age.

The young adult the endothelial cell density (ECD) is about 3000 cells/mm^2. The number of cells decreases at about 0.6% per year. The healthy endothelial cell is a regular hexagon. The average corneal diameter is 11.5 mm vertically and 12 mm horizontally. It is 540 µm thick centrally on average, and thicker towards the periphery [17].

Specular microscopes used today are based on design suggested by Maurice., *et al*. The modern noncontact specular microscope to study corneal endothelium employ automated interfacing for obtaining image through a discrete focusing technology [8]. Considering the huge diabetic population in India and insufficiency of literature in India [7], we proposed a study to evaluate corneal endothelial changes using specular microscope in patients of type 2 DM.

Aim of the Study

Moreover, we aimed to determine the association of corneal morphological features with the general characteristics and laboratory data of diabetic patients, including disease duration, HbA1c, type of medications.

Our study aimed to compare the morphological characteristics of corneal endothelial cells in type 2 diabetic patients and age-matched healthy subjects by specular microscopy.

Materials and Methods

A case-control study tertiary health care centre, with a total of 50 diabetic patients and 50 healthy controls were enrolled in the study. All participants underwent a complete ophthalmological examination. Corneal endothelial measurements were performed using a non-contact specular microscopy EM-4000 SN 217409 By Tomey corporation. Laboratory data including serum fasting glucose, HbA1c levels. Specular microscopy findings and CCT of all patients were compared after approval by institutional ethical committee and Research Committee. Patients who qualified the inclusion criteria were enrolled for the study after taking a written informed consent.

Inclusion criteria:

- All known cases of type II DM along with non-diabetic age matched normal population coming to ophthalmology out patient department.
- Age above 20 years.

Exclusion criteria:

- Non consenting patients.
- Use of contact lenses.

- Presence of dry eye disease.
- Other coexisting ocular morbidities including corneal scar, cataract or glaucoma.
- History of ocular trauma.
- History of prior ocular surgeries.

Methodology

After taking written informed consent, the patient was evaluated. In total, 50 diabetic patients and 50 healthy, control participants were concomitantly enrolled in the study. All participants underwent a complete ophthalmic evaluation, including Slit-lamp biomicroscopic examination.

The patients who were diagnosed with type 2 diabetes and under follow-up in our hospital were included in the study. The diagnosis of diabetes was based on the ADA criteria; fasting blood glucose ≥ 126 mg/dl on two separate occasions, random blood glucose (RBG) ≥ 200 mg/dl with symptoms or 2-h plasma glucose ≥ 200 mg/dl.

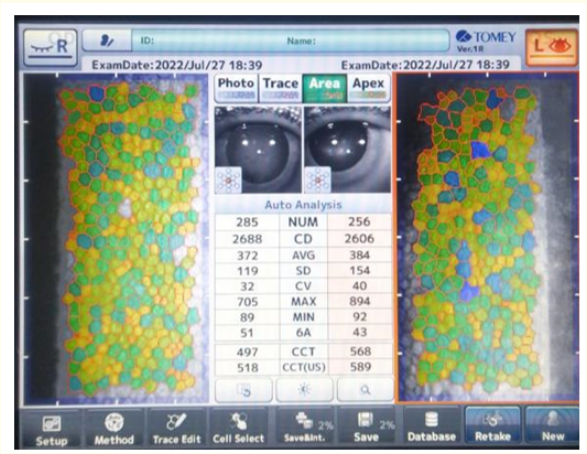

Figure 1: *Specular microscopy of a non-diabetic patient with normal ECD, CV, 6A and CCT.*

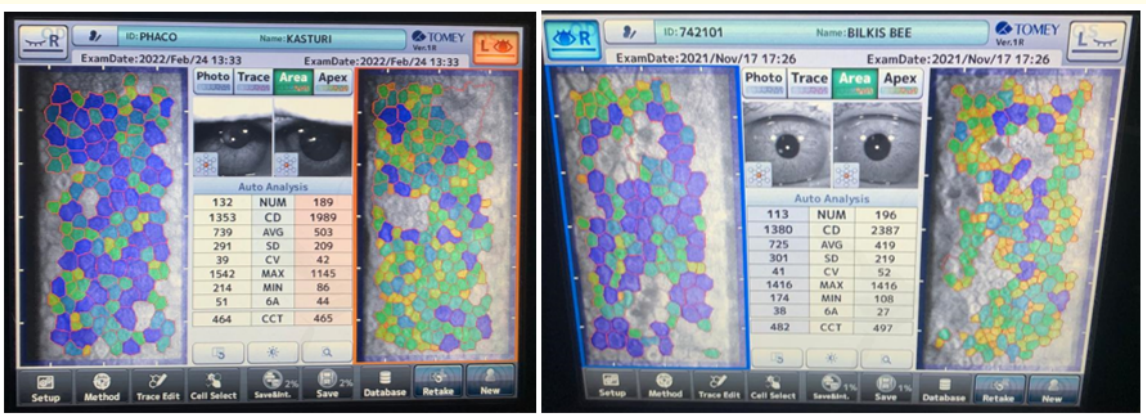

Figure 2: *Specular microscopy pictures showing decrease in endothelial cell count, increase in hexagonality and coefficient of variability in cell sizes.*

Both eyes of all participants were examined. ECD, average cell size, percentage of hexagonal cells (6A), CV in cell size, and CCT of all patients were measured using a noncontact specular microscopy.

Statistical analysis

- Statistical analysis was performed using SPSS 22.
- The one sample Kolmogorov-Smirnov test was employed to determine the normal distribution.
- Non-normally distributed data was analysed using non parametric tests. Descriptive statistics was calculated for quantitative variable and categorical variables.
- Pie or bar diagrams were used for appropriate variables.
- Paired t test and chi square test were applied.

p value < 0.05, then it was considered statistically insignificant result.

			Group		Total
			DM	Control	
Sex	F	Count	25	32	57
		%	50.0%	64.0%	57.0%
	M	Count	25	18	43
		%	50.0%	36.0%	43.0%
Total		Count	50	50	100
%		100.0%	100.0%	100.0%	
Chi-Square Tests					
	Value	Df	P Value	Result	
Pearson Chi-Square	1.108[a]	1	0.292	Non Sig	

Table 1: *Shows sex distribution in diabetes vs control group.*

Results

In total, 100 eyes of 50 patients (25 male, 25 female) with type 2 diabetes mellitus were included in the study. In the control group, 100

Crosstab					
			Group		Total
			DM	Control	
Age Group	45-60 Years	Count	20	15	35
		%	20.0%	30.0%	23.3%
	61-75 Years	Count	49	22	71
		%	49.0%	44.0%	47.3%
	76-90 Years	Count	31	13	44
		%	31.0%	26.0%	29.3%
Total		Count	50	50	100
%		100.0%	100.0%	100.0%	
Chi-Square Tests					
	Value	df	P Value	Result	
Pearson Chi-Square	1.889[a]	2	0.389	Non Sig	

Table 2: *Shows age distribution in diabetes vs control group.*

eyes of 50 (32 male, 18 female) healthy cases with normal fasting blood glucose.

Group		N	Mean	Std. Deviation	T Test	P value	Result
Cell density	DM	50	1855.02	284.136	-9.935	0.000	Sig
	Control	50	2390.12	359.097			
Coefficient of variation	DM	50	35.58	3.108	-6.716	0.000	Sig
	Control	50	40.06	5.028			
Polymegathism	DM	50	49.83	5.158	7.003	0.000	Sig
	Control	50	43.14	6.174			

Table 3: *Shows the endothelial cell density (CD), hexagonal cell ratio (6A), coefficient of variation (CV) in diabetic vs control group.*

On Sex distribution in our study, (57%) were males and (43%) were females. (50%) females and (50%) males were in the diabetic group and (64%) females and (36%) males were in the control group.

Dependent Variable	Pairwise comparison		Mean Difference	Std. Error	P Value	Result
Cell Density	<=5 Years	6-15 Years	113.291	98.206	0.657	Non Sig
		16-25 Years	310.408*	94.229	0.007	Sig
		26-35 Years	583.029*	106.746	0.000	Sig
	6-15 Years	16-25 Years	197.118*	54.637	0.003	Sig
		26-35 Years	469.738*	74.168	0.000	Sig
	16-25 Years	26-35 Years	272.620*	68.815	0.001	Sig

Table 4: *Shows endothelial cell loss in patients with duration of diabetic disease.*

On comparing age distribution in these patients, (47.3%) were in the age group of 61 - 75 years, (23.3%) in the age group of 45 - 60 years and (29.3%) in the age group of 76 - 90 years.

Dependent Variable	Pairwise comparison		Mean Difference	Std. Error	P Value	Result
Polymegathism	<=5 Years	6-15 Years	2.483	2.169	0.663	Non Sig
		16-25 Years	1.776	2.081	0.829	Non Sig
		26-35 Years	3.867	2.358	0.361	Non Sig
	6-15 Years	16-25 Years	-0.707	1.207	0.936	Non Sig
		26-35 Years	1.384	1.638	0.833	Non Sig
	16-25 Years	26-35 Years	2.091	1.520	0.518	Non Sig

Table 5: *Comparison of polymegathism (6A) in patients with duration of diabetic disease shows no significant changes among two groups.*

On comparing, the ECD were significantly lower, while the hexagonality, the average cell size and CV%, were determined to be signifi-

Dependent Variable	Pairwise comparisons		Mean Difference	Std. Error	P Value	Result
Coefficient of Variation	<=5 Years	6-15 Years	-0.601	1.319	0.968	Non Sig
		16-25 Years	0.224	1.265	0.998	Non Sig
		26-35 Years	0.371	1.433	0.994	Non Sig
	6-15 Years	16-25 Years	0.825	0.734	0.675	Non Sig
		26-35 Years	0.972	0.996	0.763	Non Sig
	16-25 Years	26-35 Years	0.147	0.924	0.999	Non Sig

Table 6: *Comparison of coefficient of variability in patients with diabetes with duration of disease.*

cantly higher in diabetic patients than in healthy controls.

Variable	Medications	N	Mean	Std. Deviation	T Test	P Value	Result
Cell density	HAI	22	1690.95	211.209	5.622	0.000	Sig
	OHA	28	1973.83	271.912			
Coefficient of variation	HAI	22	35.26	3.116	0.870	0.387	Non-Sig
	OHA	28	35.81	3.109			
Polymegathism	HAI	22	48.90	5.560	1.537	0.128	Non-Sig
	OHA	28	50.50	4.784			

Table 7: *Comparison of patients with diabetes on insulin or oral hypoglycemic drugs.*

There was a statistically significant positive correlation between ECD loss vs. duration of DM.

HbA1C		N	Mean	Std. Deviation	T Test	P Value	Result
Cell density	5.7-6.4%	3	2132.00	133.101	1.732	0.086	Non Sig
	>=6.5%	97	1846.45	283.576			
Coefficient of variation	5.7-6.4%	3	34.33	5.508	-0.704	0.483	Non Sig
	>=6.5%	97	35.62	3.046			
Polymegathism	5.7-6.4%	3	53.33	0.577	1.197	0.234	Non Sig
	>=6.5%	97	49.72	5.200			

Table 8: *Comparison of patients with diabetes with increasing HbA1c.*

There is no statistically significant correlation between polymegathism with duration of DM.

Showing no significant change in coefficient of variation with increasing duration of diabetes.

Showing significant endothelial cell loss in patients on oral hypoglycemic drugs (p-value = 0.00). And no significant changes in CV (p value = 0.387) or Polymegathism (p value = 0.128).

Showing no significant changes in ECD (p value = 0.086), CV (p value = 0.483) or Polymegathism (p value = 0.234).

Discussion

The cornea is incapable of mitosis, and corneal endothelial cells have no regenerative capacity. For that reason, we can suggest that an elongation in diabetic duration, the decrease in ECD cannot be restored. The only compensatory mechanism is the increased cellular pleomorphism and a decrease in the percentage of hexagonal cells. The fact that the cornea is transparent does not mean that the endothelium is normal. It may be useful for early diagnosis to direct patients with endothelial problems to nephrologists for clinical evaluation of nephropathy.

The corneal endothelium can be evaluated by specular microscopy. In respect with the data, we obtained at the end of this study, diabetes mellitus reduces corneal endothelial functional reserve. Reduced endothelial reserve increases the risk of corneal endothelial damage in intraocular surgery.

In this study, we determined a significant decrease in ECD values (standard deviation of 284.13) in diabetic patients compared with the values in age-matched controls (standard deviation of 359.09) irrespective of control of diabetes, and there was a negative correlation between ECD and diabetes duration as patients with more duration of diabetic. Similarly, El-Agamy., *et al*. [3], Sudhir., *et al*. [7], Islam QU., *et al*. [1], Choo., *et al*. [10], Lee., *et al*. [14] reported that ECD was significantly lower in diabetic patients than in controls. But in a prospective study by Storr-Paulsen., *et al*. [4] reported that there were no significant differences between type 2 diabetic patients with good glycemic control and nondiabetic control subjects in ECD.

We also identified correlation between average cell size and Coefficient of variation as there are increase in cell sizes and hexagonality in diabetics, Similar to our results, Lee., *et al*. [14] also reported hexagonality in diabetic eyes with thicker corneas compared with the same parameters in healthy controls. Moreover, they also determined an augmentation in corneal morphological abnormalities in patients with a diabetic duration of over 10 years [3]. El-Agamy., *et al*. reported in his study that CV was significantly higher in diabetic patients, but the differences in hexagonal cell percentage and CCT were not significantly different between diabetic patients and healthy controls. On the other hand, Choo., *et al*. [10] reported that hexagonality significantly decreased, while average cell size and CV increased significantly in diabetic patients. However, they determined no significant alteration in the CCT of patients with diabetes. On the Contrary, Sudhir., *et al*. [7], Islam QU., *et al*. [1] and Storr-Paulsen., *et al*. [4] reported that there was no significant difference between groups regarding hexagonality % or CV of cell size.

We also compared laboratory parameters like HbA1c. There were no significant differences between type 2 diabetic patients and nondiabetic control subjects in ECD, CV or polymegathism.

We also compared among diabetic group who are on Insulin or oral hypoglycemic drugs. There were significance in Endothelial cell loss in group using oral hypoglycemic drugs. We did not find any significance changes in CV and hexagonality %.

We could not find HbA1c and medications related comparison in endothelial cell loss, CV or polymegathism in any other study.

Age is defined as the most important factor in evaluating corneal morphology, and it is known that with age, ECD decreases, and the corneal endothelium compensate by increasing the size. In this study, the diabetic and control groups were age matched. However, in subgroups of diabetic patients as we compared ECD loss with duration of disease, HbA1c and use of oral hypoglycemic drugs or insulin, age could not be matched, which may have affected the results and is one of the limitations of this study.

Additionally, the risk of corneal decompensation should be kept in mind in diabetic patients as the severity of the disease increases. In diabetic patients with diabetic retinopathy and with nephropathy, caution should be taken in terms of endothelial decompensation. To minimize endothelial damage during cataract surgery and other surgeries, if necessary, endothelial protective manoeuvres should be performed, and preoperative specular microscopy findings should be carefully examined in these patients.

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