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Original Article

Ophthalmology Section

Corneal Endothelial Morphology and Central Corneal Thickness Changes in Type 2 Diabetes Mellitus using Specular Microscopy and Ultrasonic Pachymetry: A Cross-sectional Comparative Study

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is a metabolic condition characterised by chronic hyperglycaemia, causing raised blood glucose levels which result in microvascular and macrovascular disorders and may introduce ocular manifestations including changes in corneal Endothelial Cell Density (ECD), corneal thickness, and intraocular pressure. It is clinically important to analyse the corneal endothelial status in patients with type II DM as preoperative corneal endothelial cell dysfunction may cause more corneal endothelial cell damage postoperatively leading to corneal decompensation. With the advent of precise and better measurement tool Central Corneal Thickness (CCT) and corneal endothelial morphology measurement has become more accurate.

Aim: To compare corneal endothelium cell density, polymorphism, polymegathism and CCT in type 2 DM with age-matched, non diabetic control subjects using CEM-530 Specular microscope and ultrasonic pachymeter Tomey SP-100.

Materials and Methods: This cross-sectional, comparative study was conducted at Eye Department at ESI Post Graduate Institute of Medical Sciences and Research, Basaidarapur, Delhi, India, from October 2018 to November 2019 on a total of 150 patients. Seventy five known type 2 DM patients were enrolled in diabetic group (case group) and 75 non diabetic, age-matched subjects were enrolled as control group. Cases were classified under three major groups, namely on the basis of duration of DM and severity of Diabetic Retinopathy (DR) and glycaemic control {glycosylated haemoglobin (HbA1c) level}. All the findings were endorsed on a predesigned performa. Statistical Package for Social Sciences (SPSS) version 17.0 was used for analysis of data.

Results: Among the 150 patients evaluated, type 2 DM patient's corneas (540.51±32.578 μ) were thicker as compared to control group 517.51±22.155 μ (p-value <0.001). The mean ECD of control and diabetic group patients was 2723.75±287.253 cells/mm^2 and 2716.11±296.081 cells/mm^2, respectively, found insignificant (p-value=0.821). The mean Coefficient of Variation (CV) of cell area of control and diabetic patients was 28.87±3.950 and 29.85±4.027, respectively, and was significant (p-value=0.034). The mean percentage of endothelial Hexagonal cells (HEX%) of control and diabetic patients were 67.39±6.419 and 67.41±5.493, respectively and was non significant (p-value=0.985). Thus, statistically significant difference was found with CCT (p-value <0.001) and CV (p-value=0.034) but not with ECD and hexagonality, between control and diabetic eyes. There was a correlation between CCT, CV, HEX% and ECD with duration of DM2 but it was statistically insignificant. There were higher CCT, CV and HEX% and lower ECD in >10 years of duration of diabetes mellitus than in patients with duration of diabetes mellitus ≤10 years. There was a correlation of CCT, CV, ECD and HEX with HbA1c level. There were significant higher CCT and CV values in >7% HbA1c level group than in group with ≤7% HbA1c level. There was also increased ECD in >7% group, but it was found to be insignificant. Percentage of hexagonality in >7% HbA1c level group was lower than in group with ≤7% HbA1c level, but found insignificant. There were higher values of CCT and CV in Non Proliferative Diabetic Retinopathy (NPDR) subgroup compared to Proliferative Diabetic Retinopathy (PDR) and no Diabetic Retinopathy group.

Conclusion: The present study documented that DM has considerable effects on all the layers of the cornea especially endothelial layer, causes reduction of ECD and increased CV. Diabetic cornea has increased CCT and lower percentage of hexagonal cells than normal subjects. In addition, there is a correlation between the changes in corneal parameters like ECD, CV, HEX%, CCT with the duration of DM and severity of DR and glycaemic control {glycosylated haemoglobin (HbA1c) level}.

Keywords: Coefficient of variation, Diabetic retinopathy, Endothelial cell density, Glycosylated haemoglobin, Hexagonality

INTRODUCTION

The Diabetes Mellitus (DM) is a metabolic condition characterised by chronic hyperglycaemia subsequently causing raised blood glucose levels which results in microvascular and macrovascular disorders and may introduce ocular manifestations including changes in corneal Endothelial Cell Density (ECD), corneal thickness, and intraocular pressure [1]. The prevalence of diabetes is estimated to be about 6.4% worldwide, and in the past two decades alone there has been a dramatic increase in the diagnosis of type II DM [2].

The DM is one of the chief causes of blindness globally and can affect eye leading to ocular problems including Diabetic Retinopathy (DR), cataracts, keratopathy, glaucoma [3]. Clinical evidence shows that patients with type II diabetes present alterations, such as increased epithelial fragility and recurrent erosions, reduced sensitivity, impaired wound healing, altered epithelial barrier function, and persistent stromal oedema after intraocular surgical procedures [4,5].

Corneal endothelial cells are organised in a monolayer. At birth, Corneal Endothelial Cell Density (CED) ranges from 4000-5000 (cells/mm^2).

With age, it regresses at a rate of 0.3-0.6% per year and reaches approximate range of 2000-3000 cells/mm^2 in a normal adult eye [6,7]. CED decreases with age, trauma, refractive surgery, intraocular surgery, glaucoma, corneal dystrophies and DM [7]. Central Corneal Thickness (CCT) is another important parameter for corneal health as the Intraocular Pressure (IOP) depends on corneal thickness and CCT must be taken into consideration in evaluating glaucoma patients or suspects [8]. Human corneal endothelial cells do not regenerate after injury but heal through their hyperplasia and mobilisation [9].

Various studies suggest that, in diabetes cornea, there is decrease in ECD and polymorphism (cell shape variation) means decrease in the percentage variation) 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) along with increased CCT [1,10-12]. Few studies suggest clinical importance of CCT assessment for diagnosing intraocular pressure pathologies and ECD evaluation to determine the compromise of the endothelial barrier function [13,14].

The hypothesis of the study was that type 2 DM causes decrease reduction of ECD, along with lower percentage of hexagonality (polymorphism), increased CV (polymegathism) and increased CCT as compared to age matched non diabetic subjects. There is paucity of Indian studies carried out in relation with this topic, so this study is an attempt to fill the gap. The primary objective of this study was to compare corneal ECD, CV, percentage of endothelial hexagonal cells (HEX%) and CCT in type 2 DM with age-matched, non diabetic control subjects using CEM-530 Specular Microscope and ultrasonic pachymetry Tomey SP-100.

The secondary objective of the study was to assess correlation between the changes in above mentioned corneal parameters and the duration of DM, severity of DR and glycaemic control {glycosylated haemoglobin (HbA1c) level}.

MATERIALS AND METHODS

This cross-sectional, comparative study was conducted at Eye Department at ESI Post Graduate Institute of Medical Sciences and Research, Basaidarapur, Delhi, India, from October 2018 to November 2019. Patients with ages between 40-70 years of either gender who were diagnosed to have DM were recruited in the study, after approval by Ethical Review Committee of Hospital (DM/A91-9/14/17/IEC/2012-PGIMSR) (PART-II). Informed and written consent was obtained from each subject before enrollment.

Sample size calculation: To calculate the number of participants needed for this study, the significance level was set at 95% (α=0.05), and the power of the test was set at 80% with a type II error (β) of 0.20. A previous study by El-Agamy A and Alsubaie S, found that mean ECD in the control group was 2660.1±515.5 [15]. Assuming that ECD width decreases in type II diabetic patients, sample size of 35 eyes per group was calculated:

$n=(\sigma1^2+\sigma2^2).(Z\ 1-\alpha/2+Z\ 1-\beta)^2/(M1-M2)^2$

$=(515.52+515.5^2).(1.96+1.282)^2/(399.02*399.02)$

$=(265740.3+265740.3)*10.51/159213=65.08$

Where,

$Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g., for a confidence level of 95%,

α is 0.05 and the critical value is 1.96),

Z_β is the critical value of the normal distribution at β (e.g., for a power of 90%, β is 0.1 and its critical value is 1.282)

σ 1 and σ 2 are the standard deviations of the two groups and M1 and M2 are the means of two groups.

After calculating minimum sample size for present study to get the statistically significant results, minimum participants required for this study were 65 and hence:

Diabetic group (n=75 cases, 150 eyes): Cases were classified under three major groups, namely on the basis of duration of DM, severity of DR and glycaemic control (glycosylated haemoglobin level):

- Duration of DM with ≤10 years of the disease or >10 years of the disease.
- Presence or absence of Diabetic Retinopathy (DR) and patients having DR were further classified into three subgroups:

  Patients having no DR

  Patients with Non Proliferative Diabetic Retinopathy (NPDR)

  Patients with Proliferative Diabetic Retinopathy (PDR)
- Glycosylated Haemoglobin (HbA1c) levels of the patients ≤7.0% or >7.0% at the time of presentation (American Diabetes Association) [16].

Control group (n=75 subjects, 150 eyes): Age-matched non diabetic subjects formed the group.

All patients were recruited by non randomised convenience sampling method.

Inclusion criteria: Cases were patients aged between 40-70 years of either gender, and diagnosed case of type 2 DM. Diagnosis of DM was based on criteria of the American Diabetes Association [16]. Controls were age-matched non diabetic subjects.

Exclusion criteria: Patients with presence of history of past ocular or intraocular surgery, corneal disease (dystrophies) or any signs of previous corneal disease (corneal opacity), ocular inflammation or trauma, previous retinal photocoagulation laser and anti-vascular endothelial growth factor therapy injection, contact lens wearer, glaucoma, pterygium, entropion or trichiasis, rheumatoid arthritis and systemic lupus erythematous that are known to impair tear function were excluded from the study.

Age, gender, duration of diabetes, level of HbA1c, severity of DR, current medical treatment, other systemic co-morbidities such as rheumatoid arthritis, systemic lupus erythematous that are known to impair corneal morphology along with impaired tear function.

Study Procedure

All subjects underwent following complete ophthalmic examination which included, visual acuity assessment using Snellen chart following refractive acceptance. Intraocular pressure (mmHg) measurement using Goldmann applanation tonometer, slit-lamp biomicroscopy and Fundus examination by 90D biomicroscopy and indirect ophthalmoscope.

- The corneal endothelial parameters: ECD (cell/mm^2), hexagonality (HEX%), CV measured using specular microscope (CEM-530; NIDEK) by a single examiner.
- Central Corneal Thickness (CCT) (µm) was measured by ultrasonic pachymetry (Tomey SP-100). Measurements were taken three times in the centre of cornea. An average of three readings was used for final analysis.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 17.0 was used for analysis of data. Continuous variables, presented as mean±SD, and categorical variables, presented as absolute numbers and percentage. Age and corneal parameters (ECD, CCT, CV and HEX%) compared using unpaired t-test in diabetic and control group. Also, for comparison in between subgroup of diabetic patients on HbA1c level done by using Analysis of Variance (ANOVA) test. Comparison among gender (male and female) done by using Chi-square test. Corneal parameters (ECD, CCT, CV and HEX%) was correlated using Pearsons correlation test. Independent t-test is used to compare the variables between the duration of diabetes disease <10 years and >10 years. Right and left eye parameters were compared using paired t-test in diabetic group and control group separately. The level of significance was set at p-value ≤0.05.

RESULTS

A total of 150 subjects that met the inclusion criteria and exclusion criteria were included in the study. As shown in [Table/Fig-1], mean age was 52.8±8.77 years in control group and 53.25±8.36 years in diabetic group (p-values=0.746; unpaired t-test).

Demographic data	Control group (n=75)	Diabetic group (n=75)	p-values (Unpaired t-test)
Age (years) (mean±SD) (range)	52.8±8.77 (40-69)	53.25±8.36 (40-67)	0.746

[Table/Fig-1]: Distribution of mean age (years) between controls and diabetics patients.
p-value ≤0.05 was considered as statistically significant

In the present study population, there were 40 (53.3%) male with mean age of 53.4±8.65 years and 35 (46.7%) female with mean age of 53.09±8 years in diabetic group. A 42 (56.0%) male with mean age of 51.31±7.38 years and 33 (44.0%) female with mean age of 54.7±9.94 years in control group, statistically non significant (p-value=0.743) [Table/Fig-2].

Gender	Control group	DM patients (eyes)
Male	42 (56.0%)	40 (53.3%)
Female	33 (44.0%)	35 (46.7%)
p-value (Chi-square test)	0.743	

[Table/Fig-2]: Sex distribution of subjects.

As shown in [Table/Fig-3], 102 eyes found to have no diabetic changes in retina, 40 eyes had NPDR while eight eyes had PDR. An analysing duration of DM2, 55 patients (110 eyes) had history of <10 years and 20 patients (40 eyes) had history of >10 years. Among the 75 diabetic patients, 37 patients (74 eyes) found to have HbA1c levels less than 7% and 38 patients (76 eyes) had HbA1c levels more than 7%.

Clinical data	Diabetic retinopathy			DM duration (years)		HbA1c	
	No DR (n, %)	NPDR (n, %)	PDR (n, %)	<10 (n, %)	>10 (n, %)	<7% (n, %)	>7% (n, %)
Eyes (n=150)	102 (68)	40 (26.66)	8 (5.33)	110 (73.3)	40 (26.66)	74 (49.33)	76 (50.66)

[Table/Fig-3]: Distribution of diabetic patients (eyes) in subgroups.
DR: Diabetic retinopathy; NPDR: Non proliferative DR; PDR: Proliferative DR; HbA1c: Haemoglobin A1c

Spectrometry and Pachymetry Data Evaluation

The mean CCT of control and diabetes group patients was 517.51±22.155 microns and 540.51±32.578 microns, respectively and was significant (p-value <0.001). The mean ECD of control and diabetes group patients was 2723.75±287.253 cells/mm^2 and 2716.11±296.081 cells/mm^2, respectively and difference was not significant (p-value=0.821). The mean CV of control and diabetes group patients was 28.87±3.950 and 29.85±4.027, respectively and difference was found significant (p-value=0.034). The mean HEX% of control and diabetes group patients was 67.39±6.419 and 67.41±5.493, respectively and difference was not significant (p-value=0.985) [Table/Fig-4]. The ECD values were found to be on higher side in control eyes than diabetic eyes but this difference was statistically insignificant. Likewise, statistically insignificant difference was noted with HEX% but with slightly increased value in diabetic patients.

Duration of DM and corneal parameters: Eyes with DM duration of ≥10 years showed higher CCT (543.70±25 µm), patients with DM ≤10 years (539.35±34 µm), statistically non significant (p-value=0.471). ECD was higher in patients with DM ≤10 years (2722.48±28 cells/mm^2) (p-value=0.663). The CV was higher in patients with DM ≥10 years group (29.90±4.2) as compared to patient with DM ≤10 years group (29.84±3.9) (p-value=0.932). No statistical significance in HEX% between the two groups (p-value=0.161) [Table/Fig-5].

HbA1c and and corneal parameters: The CCT was higher and significant among patients with HbA1c >7% (546.58±30.10 µm) as compared to patients with HbA1c ≤7% group (534.27±34.02 µm) (p-value=0.020). The HEX% (p-value=0.681) and ECD (p-value=0.703) between the two groups was statistically insignificant but CV between the two groups was statistically significant (p-value=0.006) [Table/Fig-6]. There was higher values of ECD in eyes with HbA1c >7% than HbA1c <7% eyes, but this result was statistically insignificant.

Diabetic retinopathy and corneal parameters: Comparison of CCT among diabetic patients using One-way ANOVA test showed that the mean value CCT in eyes with NPDR (554.20±26.912 µ) was highest followed by eyes not having any DR (535.60±34.001 µ) and least in PDR eyes (534.63±20.340 µ), difference was found

Group	CCT (µm)			ECD (cell/mm^2)			CV			Hexagonality (%)		
	Total	RE	LE	Total	RE	LE	Total	RE	LE	Total	RE	LE
Control	517.51± 22.155	517.08± 22.88	517.95± 21.55	2723.75± 287.253	2726.07± 290.85	2721.44± 285.55	28.87± 3.950	29.01± 3.84	28.73± 4.08	67.39± 6.419	67.07± 6.48	67.72± 6.38
Diabetic	540.51± 32.578	539.07± 32.54	541.95± 32.77	2716.11± 296.081	2720.89± 263.45	2711.32± 327.2	29.85± 4.027	29.92± 3.79	29.79± 4.28	67.41± 5.493	67.44± 5.71	67.37± 5.3
p-value	<0.001	<0.001	<0.001	0.821	0.909	0.84	0.034	0.148	0.125	0.985	0.709	0.718

[Table/Fig-4]: Comparison of corneal parameters in control and diabetic patient.
Test applied-Independent t-test
CCT: Central corneal thickness; CV: Coefficient of variation cell area; ECD: Endothelial cell density

Group	CCT (µm)			ECD (cells/mm^2)			CV			Hexagonality (%)		
	RE	LE	Total	RE	LE	Total	RE	LE	Total	RE	LE	Total
≤10 years	537.8	540.8	539.3	2723.6	2721.3	2722.4	30.0	29.6	29.8	66.8	67.2	67.0
	40±34	50±35	50±34	40±24	30±32	80±28	40±3	40±4	40±3	20±5	40±5	30±5
	0.989	0.005	0.869	2.254	1.262	3.208	0.751	0.200	0.969	0.494	0.270	0.363
>10 years	542.4	544.9	543.7	2713.3	2683.8	2698.5	29.6	30.2	29.9	69.1	67.7	68.4
	50±25	50±26	00±25	50±32	00±35	80±33	00±3	00±4	00±4	50±6	50±5	50±5
	0.053	0.227	0.347	1.629	0.068	2.149	0.966	0.572	0.235	0.089	0.514	0.778
t value	-0.54	0.476	-0.723	0.149	0.437	0.436	0.439	0.502	-0.085	-1.579	0.369	-1.407
p-value	0.591	0.635	0.471	0.882	0.664	0.663	0.662	0.617	0.932	0.119	0.713	0.161

[Table/Fig-5]: Comparison between corneal parameters and duration of diabetes patients.
Test applied-Independent t-test
CCT: Central corneal thickness; CV: Coefficient of variation cell area; ECD: Endothelial cell density

Group	CCT (µm)			ECD (cell/mm²)			CV			Hexagonality (%)		
	Total	RE	LE	Total	RE	LE	Total	RE	LE	Total	RE	LE
HbA1c≤7%	534.27± 34.021	533.76± 34.54	534.78± 33.96	2706.73± 288.742	2711.38± 234.79	2702.08± 337.48	28.95± 3.888	29.08± 3.35	28.81± 4.4	67.59± 5.674	67.51± 5.89	67.68± 5.53
HbA1c>7%	546.58± 30.104	544.24± 30.01	548.92± 30.41	2725.24± 304.695	2730.16± 291.55	2720.32± 321.15	30.74± 3.453	30.74± 4.05	30.74± 3.98	67.22± 5.343	67.37± 5.61	67.08± 5.13
p-value	0.020	0.165	0.061	0.703	0.76	0.811	0.006	0.058	0.051	0.681	0.913	0.629

[Table/Fig-6]: A comparison of the mean values of CCT, ECD, CV, and hexagonality between the diabetic groups according to HbA1c% (mean±SD).
CCT: Central corneal thickness; CV: Coefficient of variation cell area; ECD: Endothelial cell density; HbA1c : Haemoglobin A1c

to be statistically significant (p-value=0.007). The mean value of ECD in eyes with NPDR (2637.05±301.580 cells/mm²) was lowest followed by PDR eyes (2744.38±299.488 cells/mm²) and highest in eyes not having any DR (2744.89±290.886 cells/mm²), found to be statistically insignificant (p-value=0.143). The mean value of CV in eyes with NPDR (31.65±4.560) was highest followed by eyes not having any DR (29.37±3.609) and least in PDR eyes (27.00±3.207), found to be statistically significant (p-value=0.001). The mean value of hexagonality in eyes with PDR (72.00±4.472%) was highest followed by NPDR eyes (67.28±5.325%) and lowest in eyes not having any DR (67.10±5.514%), found to be statistically significant (p-value=0.050) [Table/Fig-7].

Group	CCT	ECD	CV	Hexagonality (%)
No DR (n=102)	535.60± 34.001	2744.89± 290.886	29.37± 3.609	67.10± 5.514
NPDR (n=40)	554.20± 26.912	2637.05± 301.580	31.65± 4.560	67.28± 5.325
PDR (n=8)	534.63± 20.340	2744.38± 299.488	27.00± 3.207	72.00± 4.472
p-value	**0.007**	**0.143**	**0.001**	**0.050**

[Table/Fig-7]: Comparison of corneal parameters with DR grading.
CCT: Central corneal thickness; CV: Coefficient of variation cell area; DR: Diabetic retinopathy; ECD: Endothelial cell density; NPDR: Nonproliferative DR; PDR: Proliferative DR

The Pearson correlation coefficient analysis showed that DM duration had no significant correlation with CCT, CV, HEX% and ECD.

Correlation: The Pearson's correlation coefficient analysis showed that DM duration had no significant correlation with CCT, CV, HEX% and ECD. The Pearson's correlation coefficient between HbA1c value and CCT was significant (p-value <0.001). Similarly, Pearson's correlation coefficient between HbA1c value and CV was significant (p-value=0.003). The Pearson correlation coefficient between HbA1c value and ECD (p-value=0.505) was insignificant. The corneal hexagonality had negative Pearson correlation coefficient with HbA1c for both eyes and this was statistically non significant. In case of diabetic patients there was a weak correlation between CCT and CV; and CV and ECD. HEX% was significant (p-value=0.012) with respect to CCT in diabetic patients. In control group, there was a negative correlation between CV and HEX% and there was a positive correlation ECD and HEX% [Table/Fig-8-11].

Parameters	Pearson's correlation	p-value
CCT RE	0.055	0.641
CCT LE	0.034	0.773
CV RE	-0.056	0.631
CV LE	0.026	0.825
ECD RE	-0.062	0.595
ECD LE	-0.107	0.359
HEX% RE	0.171	0.141
HEX% LE	0.088	0.455

[Table/Fig-8]: Correlation between corneal parameters and duration of Diabetes Mellitus (DM).
Test applied- Pearson's correlation test
CCT: Central corneal thickness; CV: Coefficient of variation cell area; ECD: Endothelial cell density; HEX%: Hexagonality

Parameters	Pearson correlation coefficient	p-value
CCT RE	0.502	<0.001
CCT LE	0.512	<0.001
CV RE	0.337	0.003
CV LE	0.271	0.019
ECD RE	0.078	0.505
ECD LE	0.074	0.529
HEX% RE	-0.042	0.723
HEX% LE	-0.098	0.401

[Table/Fig-9]: Correlation between corneal parameters and HbA1c.
Test applied- Pearson's correlation test
CCT: Central corneal thickness; CV: Coefficient of variation cell area; ECD: Endothelial cell density; HEX%: Hexagonality

Parameters	CCT	CV	ECD	HEX%
CCT				
Pearson correlation	1	0.293	0.048	-0.204
p-value		<0.001	0.556	0.012
CV				
Pearson correlation	0.293	1	0.270	-0.392
p-value	<0.001		0.001	<0.001
ECD				
Pearson correlation	0.048	0.270	1	-0.084
p-value	0.556	0.001		0.305
HEX%				
Pearson correlation	-0.204	-0.392	-0.084	1
p-value	0.012	<0.001	0.305	

[Table/Fig-10]: Correlation of corneal changes among diabetic cases.
Test applied- Pearson's correlation test
CCT: Central corneal thickness; CV: Coefficient of variation cell area; HEX%: Hexagonality; ECD: Endothelial cell density

Parameters	CCT	CV	ECD	HEX%
CCT				
Pearson correlation	1	-0.122	0.006	-0.018
p-value		0.136	0.943	0.823
CV				
Pearson correlation	-0.122	1	0.106	-0.609
p-value	0.136		0.199	<0.001
ECD				
Pearson correlation	0.006	0.106	1	0.341
p-value	0.943	0.199		<0.001
HEX%				
Pearson correlation	-0.018	-0.609	0.341	1
p-value	0.823	<0.001	<0.001	

[Table/Fig-11]: Correlation of corneal changes among non diabetic control group.
Test applied- Pearson's correlation test
CCT: Central corneal thickness; CV: Coefficient of variation cell area; HEX%: hexagonality; ECD: Endothelial cell density

DISCUSSION

The DM is a chronic metabolic disorder. Corneal endothelium of diabetic cornea may suffer from many morphological changes. It is

clinically important to analyse the corneal endothelial cell morphology in patients with type 2 diabetes, undergoing any intraocular surgical procedure like cataract or glaucoma surgeries. Postoperative corneal endothelial cell loss leads to corneal decompensation. This further leads to corneal endothelial cell transplantation. Thus, highlighting the importance of preoperative measurements of CCT, CV, ECD and HEX%.

In the present study, statistically significant difference was found with CCT (p-value <0.001) and CV (p-value=0.034) but not with ECD and hexagonality, between control and diabetic eyes. Higher values of CCT and CV in diabetic eyes were witnessed in this study. ECD values (p-value=0.821) were found to be on lower side in diabetic eyes than control and differences were statistically insignificant. Likewise, statistically insignificant differences were noted with HEX% (p-value=0.985) but with slightly increased value in diabetic patients.

In a similar study done by Schultz RO et al., 46 corneas of 25 type 2 DM patients with duration of >10 years were examined by specular microscopy [17]. There was no difference in ECD and CCT but revealed a considerably higher CV and reduction in the percentage of hexagonal cells, in comparison non diabetic population (n=21). Similarly in the present study, it was found that there was no significant difference in the ECD (p-value=0.821) and significant difference in the CV (p-value=0.034) in type 2 DM patients in comparison to control subjects. But to the contrary, difference in the HEX% (p-value=0.985) was found insignificant. Schultz RO et al., also showed that the coefficient of variation has a significant inverse relationship with the frequency of hexagonal cells and the figure coefficient [17]. Present study also found same correlation between CV and HEX% (Pearson's Correlation=-0.392, p-value <0.0001).

The underlying reason of this difference in results in the percentage of hexagonal cells could be dissimilarity in the duration of diabetes in case arm. In present study both >10 years and <10 years of duration of disease patients were taken while in Schultz RO et al., study only >10 years of duration of disease patients were included [17].

Larsson L et al., studied 60 known patients of type 2 DM and they found no difference in cell density among type 2 DM patients and control groups [18]. Type 2 DM patients had decreased HEX%, increased CV and increased CCT but they did not differ significantly from controls. The type II diabetics were older than the type I diabetics, and the older control group showed changes similar to those seen in the diabetics; these changes were presumably associated with aging. In contrast to this study, findings were significantly different in CCT and CV among type 2 DM patients and control groups, in present study. But HEX% was found lower in type 2 DM patients as in Larsson L et al., study [18].

Lee JS et al., studied the differences of corneal morphological parameters in DM patients compared with age matched, healthy control subjects [18]. The diabetic subjects had greater CCT, reduced ECD and hexagonality, and more CV of the corneal endothelium than the control. Lee JS et al., found thicker corneas with the mean CCT significantly higher in diabetic (588±272.7 µm) than in the control group (567±873.8 µm) (p-value <0.05) and more irregular cell sizes with the mean value of CV which was significantly higher in diabetics (38.2±0.4) than in the control group (35.4±0.6) (p-value <0.05), as in present study [19]. Lee JS et al., demonstrated significantly less mean ECD in diabetics (2577±2727.3 cell/mm^2) than in the control group (2699.9738.7 cell/mm^2) (p-value <0.05) and significantly higher mean value of hexagonality for diabetics than for normal persons (p-value <0.05) [19].

Inoue K et al., documented a significant reduction in ECD of diabetic corneas (2493±330 cells/mm^2) compared to controls (2599±278 cells/mm^2) (p-value=0.016) [20]. The CV in cell area was significantly higher in the diabetic group (37.2±6.0) than in the control group (35.4±5.0). There was no significant difference between the percentages of hexagonal cells and CCT in the diabetic group (56.1±8.5% and 538±36 µ, respectively) and the control group, (56.7±6.3% and 537±38 µ, respectively). The present study, found reduction in ECD of diabetic corneas (2493±330 cells/mm^2) compared to controls, but statistically insignificant (p-value=0.821) along with significant results in CV (p-value=0.034) as this study. This study also concluded significant difference in terms of CCT (p-value=<0.001) and insignificant difference in terms of HEX % (p-value=0.718) between diabetic and control group.

In 1999, Roszkowska AM et al., evaluated 23 type 2 DM patients for CCT, ECD, CV and HEX% [13]. They found significant difference in reduction of the mean ECD of 5% in type 2 DM patients. Roszkowska AM et al., found that the CCT was significantly higher with p-value <0.05 in the type 2 DM group [13]. Present study also demonstrated the significant difference in the CCT in type 2 DM patients in comparison to control subjects (p-value=<0.001)

In another study done by Claramonte PJ et al., on 953 non diabetic patients and 47 diabetic patients concluded that diabetic cornea were thicker (mean CCT=571.96±26.81 µm) when compared with non diabetic patients (544.89±35.36 µm) with p-value <0.001) [21]. This results were in concordance with present study findings.

Su DH et al., the Singapore Malay eye study, examined the correlation of diabetes with central CCT in 3239 Malay adults [22]. They found significantly thicker diabetes cornea than in those without diabetes (547.2 µm vs 539.3 µm, p-value<0.001). In comparison to this present study, sample size was very small but end results were similar.

Choo M et al., evaluated CCT, ECD, CV and HEX% of 200 eyes of type II diabetics and 100 eyes of non diabetic control patients [23]. They reported significant increase CV (67.2±47.2% vs 58.2±43.0%, p-value <0.01) in diabetic group which is in concordance with present study findings. They also found reduced ECD (2541.6±516.4 vs 2660.1±515.5 cells/mm^2, p-value <0.01) and hexagonality (41.1%±19.6% vs 45.2%±20.6%, p-value <0.01). They also reported no significant difference in CCT (µm; 517.3±53.4 vs 510.8±71.9, p-value=0.149).

In a study done on Indian population (1191 type 2 DM patients and 121 controls) by Sudhir RR et al., observed no difference in the mean CCT, hexagonality (%), and CV of cell among cases and controls [11]. In contrast to this, in our Indian study population, we observed significant difference in the mean CCT and CV. Sudhir RR et al., showed lower mean ECD (cells/mm^2) in cases than in controls (2550.96 vs 2634.44; p-value=0.001) which is dissimilar to our study [11].

Storr-Paulsen A et al., conducted a prospective clinical study on 107 type 2 DM patients and 128 non diabetic patients to compare ECD, CV, HEX%, CCT [1]. In their study they concluded that Type II diabetic subjects did not differ from the non diabetic control subjects with regards to ECD (2578 vs 2605 cells/mm^2), HEX%, or CV, but showed significant increase in CCT (538 versus 546 µ), (p-value <0.05). Present study also concluded significant difference with regard to CCT but also to CV.

In another study done by Stella B et al., to assess ECD and CCT in 125 diabetic patients with 90 controls [24]. The mean ECD (2511±252 cells/mm^2) and mean CCT (539.7±33.6 µm) varied significantly from those the control group ECD (2713±132 cells/mm^2) (p-value <0.0001), CCT (525.0±45.3 µ) (p-value=0.003). With regards to CCT, present study also found diabetic cornea significantly thicker than control but ECD difference did not differ significantly though it was lower in diabetic cornea.

A study was in done in 2017 with CCT, ECD, CV and percentage of hexagonal cells of 57 patients (57 eyes) with DM2 and 45 controls (45 eyes). In this study it was found that ECD was significantly lower in the diabetic cornea (2,491.98±261.08 cell/mm^2) than in control group (2,629.68±293.45 cell/mm^2) (p-value=0.014). CV was higher in diabetic cornea (0.41±0.07) (p-value=0.008). The

diabetic cornea group (33.24%±10.25%) had lower percentage of hexagonal cells than the control group (34.24%±8.73%), but the difference was not statistically significant (p-value=0.603). Also, diabetic cornea (545.61±30.39 µm) was thicker than control group (539.42±29.22 µm), but not statistically significant (p-value=0.301) [15]. In contrast to this study, in present study, statistically significant thicker diabetic cornea, was found than control group (p-value <0.001) and ECD values were found lower and HEX% were found higher in diabetic group but without significant difference (p-value=0. 821 and p-value 0.985, respectively). In terms of CV, both studies found higher levels in diabetic cornea with statistically significant different from controls cornea.

Comparison between corneal parameters and duration of diabetes patients: In present study, no correlation was found between CCT, ECD, CV and HEX% values with the duration of diabetes. Comparison of the CCT (total eyes) between the two groups shows that CCT, is higher in >10 years group, ECD is higher in <10 years group, CV is higher in >10 years group, HEX% is higher in>10 years group.

On reviewing the literature, it was found that many authors and researchers concluded the same and didn't find any association between duration of disease and changes in CCT, ECD, CV and HEX%.

Inoue K et al., performed multivariate regression analysis to assess duration of type 2 DM relation to ECD that indicated that duration of type 2 DM was not significantly correlated with the ECD [20].

Choo M et al., performed Pearson correlation analysis that showed that duration of diabetes had no significant correlations with CCT, CV, hexagonality or ECD [23]. El-Agamy and Alsubaie S also observed insignificant difference for the same [15]. In their study, eyes with DM duration of ≤10 years had higher ECD (p-value=0.658), and more hexagonality than those with DM duration of >10 year (p-value=0.111). Also, they found insignificant differences in CCT (p-value=0.431) and CV (p-value=0.927) between the two groups.

Contrary to present study and other studies mention above, Lee JS et al., found CCT significantly higher for >10 years (595.9±4.2 µm) duration of diabetes than for diabetes of ≤10 years (582.2±3.7µm) (p<0.05) [19].

One of the recent study done by Stella B et al., demonstrated significant reduction of ECD by about 32 cell/mm^2 for diabetics with duration of >10 years when compared with those with duration of ≤10 years (p-value <0.05) [24]. They also found thicker cornea for diabetics with duration of >10 years (p-value >0.05).

Comparison of corneal parameters according to HbA1c: In present study, no significant correlation was found between CCT and CV with the level of HbA1c. Comparison of the CCT between the two groups showed that CCT, ECD, CV, were higher in >7% group, HEX% was higher in ≤7% group. After going deep through the literature, it was concluded that only few of the studies stated correlation between HbA1c levels and changes in CCT, ECD, CV and HEX. Contrary to present study, Larsson L et al., demonstrated no correlation between the HbA1c and CCT, ECD, CV and HEX% in type 2 DM group [18]. Similarly, Inoue K et al., performed multivariate regression analysis to assess HbA1c value relation to ECD that indicated that HbA1c was not significantly correlated with the EC [20]. Su DH et al., the Singapore Malay Eye Study found thicker cornea in diabetic patients having higher HbA1C levels (p-value <0.001) [22]. Storr-Paulsen A et al., carried out multivariate analysis that revealed lower ECD with higher HbA1c values (p-value <0.05) in diabetic group [1]. But CCT, CV and HEX% were not associated with HbA1c levels. In the diabetic group, lower cell counts were associated with higher HbA1c values (p-value <0.05). These findings are not accordance with present study result.

Yazgan S et al., also found significant difference in CCT between group HbA1c ≤7% and group HbA1c >7% (p-value <0.001) [25]. El-Agamy and Alsubaie S, in their study showed diabetic patients with HbA1c% ≤7.5 had higher, but not statistically significant, ECD (2,537.62±311.86 cell/mm^2) than those with HbA1c% >7.5 (2,458.78±216.06 cell/mm^2) (p-value=0.293) and higher hexagonality (5.58%±10.09%) than the other group (31.54%±10.17%) (p-value=0.144) [15]. The mean of CV was significantly lower (p-value=0.017) in diabetic patients with HbA1c % ≤7.5 (0.39±0.05) than in the other group (0.43±0.07). Also, there was no statistically significant difference in CCT between the two groups (p-value=0.789).

Comparison of corneal parameters with grading of DR: In present study, DR was found in 48 eyes out of which 40 eyes having NPDR and 8 eyes having PDR. The one-way ANOVA test was applied to compare among all three groups and statistical significant difference was found with CCT, CV and hexagonality but not with ECD. Values of CCT and CV among NPDR eyes were higher than PDR eyes; it may be because of number of eyes as these numbers were higher in NPDR than PDR cases. Same reason could explain hexagonality on higher side in PDR eyes than NPDR eyes. Likewise ECD values were found to be higher in eyes with PDR than NPDR eyes but this difference was statistically insignificant. Larsson L et al., studied 60 known patients of DM2 and they found the degree of retinopathy was not significantly correlated with any of the corneal parameters (CCT, ECD, CCT and HEX%) [18]. Similarly, Inoue K et al., performed multivariate regression analysis to assess grade of DR value relation to ECD that indicated that grade of DR was not significantly correlated with the ECD [20]. Lee JS et al., measured CCT of diabetic patients with normal fundus and background DR and found higher CCT values in patients with DM compared with control group [19]. Roszkowska AM et al., reported thickened central corneas and altered endothelial morphology in diabetic patients with background retinopathy compared with normal healthy subjects [13].

In addition to above mentioned studies, El-Agamy A and Alsubaie S, found no significant differences between CCT, ECD, CV, and hexagonality percentage in diabetic patients without DR, with NPDR, and with PDR (p-value=0.344, 0.806, 0.284, and 0.500, respectively) [15]. Results of this study provide a great insight into role of preoperative evaluation of corneal morphology in diabetic population, undergoing cataract surgery, glaucoma surgery, corneal transplantation surgery, pan retinal photocoagulation of diabetic retina. Increased blood sugar level in diabetes reduces the activity of sodium-potassium adenosine triphosphatase (Na-K-ATPase) of the endothelium and causes morphological and structural changes in cornea.

Limitation(s)

In present study, patients who had history of laser photocoagulation for DR were excluded due to its influence on the corneal structures. Diabetic patients who had intravitreal injections of antivascular endothelial growth factor therapy given were not included in this study. This exclusion produced a small sample size, so additional studies with increased number of subjects are required to substantiate the results. Another limitation of the study includes not taking into account possible confounding factors like smoking and corneal diameter.

CONCLUSION(S)

This study documented that type 2 DM resulted in a reduction of ECD and increased CV along with slightly increased in hexagonality. There was higher CV in the diabetic group. There was a correlation between the changes in corneal parameters like ECD, CV, HEX%, CCT with the duration of DM and severity of DR and glycaemic control {glycosylated haemoglobin (HbA1c) level}. Results of this study suggest that long lasting DM may warrant a corneal endothelium evaluation along with CCT before any intraocular surgery. Thicker central cornea associated with DM should be taken

into consideration while obtaining accurate intraocular pressure measurements in diabetic population.

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