

Corneal Endothelial Cell Density and Cell Morphology in Type 2 Diabetes Mellitus Patients: A Case Control Study

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Abstract

Evaluation & comparison of corneal endothelial cell density and cell morphology changes between type 2 diabetes mellitus patients and age matched controls.

Materials and methods: A case control study is conducted at the out patient department at S Nijalingappa Medical college HSK Hospital and Research Centre, Bagalkot from December 2019 - June 2021. A total of 130 study subjects, patients were enrolled into two groups, 65 patients known case of diabetics and 65 non-diabetic age matched controls. All patients under went complete ophthalmic evaluation along with endothelial cell parameters.

Results: The mean endothelial cell density in diabetic group was significantly reduced compared tonon diabetic. There was higher co-efficient of variation and lower hexagonal cells in diabetic group.

Conclusion: The study concludes that the endothelial cell density was lower in diabetic group compared to controls. The altered endothelial cell morphology was significantly seen in form of polymegathism and pleomorphism (hexagonality). We also found that there is some positive correlation with duration of disease and endothelial cell density in diabetic group.

Keyword: Diabetes Mellitus; Endothelial Cell Density; Co-efficient of Variation; Hexgonality.

INTRODUCTION

Diabetes is perhaps the most important noninfective epidemic that affects the world in the present millennium. It was estimated that prevalence of diabetes in adults worldwide to be 4% in 1995 and to rise to 5.4% by 2025.¹ Type 2 diabetes mellitus (T2DM) is an extended metabolic

disease which is recognized by hyper glycemia and triggered by insulin resistance and decreased insulin release.²

Diabetes mellitus is a major cause for blindness in the world. This may lead to micro and macrovascular disorders, which may introduce ocular manifestation including changes in corneal endothelial cell density (ECD), central corneal thickness and intraocular pressure.³

Corneal endothelium plays an important role in maintaining the corneal optical transparency.⁴ India bet esthereis in creased aqueo us humor glucose levels and direct ly inhibits the corneal endothelial function. In hyperglycemic state, significantly corneas wells less. The recovery rate is slower in diabetics even in euglycemic state.⁵

Corneal endothelium is a single layer, the cells are hexagonal and metabolically active. Endothelium

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is a monolayer and when viewed from the posterior side it appears as a honeycomb like mosaic structure. There is endothelial pump action which regulates water content.⁶ The corneal endothelial cells in diabetes have morphological abnormalities. Abnormalities include decrease in endothelial cell density and hexagonality, and increase in polymegathism, pleomorphism and CCT.⁷

The endothelial changes seen in diabetes appear similar to changes caused due to aging process with a higher rate of endothelial cell loss.⁸ Diabetic cornea undergoes morphological changes as explained by many studies this was established by the assessment of polyol (sorbitol aldose reductase) pathway.⁹ The intracellular accumulation of sorbitol, causes swelling of the endothelial cells. Thus, slowing down the Krebs cycle with consequent reduction in production of ATP which is necessary for function of endothelial pump. This resulting in permeability and morphological change in the diabetic corneas. Thus, it is important to assess the corneal endothelium in all type 2 Diabetes Mellitus using Specular Microscopy. It should be a part of assessment in all diabetes patients preoperatively, so that postoperative corneal complications can be predicted based upon the parameters of specular microscopy and a high-risk cornea prone for early decompensation can be identified.¹⁰ This prospective study is conducted to evaluate the corneal endothelial cell density with age-matched controls.

METHODOLOGY

Source of data

Patients of age above 50 years and of either sex attending Ophthalmology Outpatient department in S Nijalingappa Medical College & HSK Hospital, Bagalkot, Karnataka, between the period from December 2019 to June 2021 satisfying inclusion criteria.

Method of collection of data

The data for this study were collected from the subjects fulfilling the inclusion/exclusion criteria. 130 eyes of 130 patients attending the ophthalmology department at SNMC & HSK Hospital, Bagalkot, Karnataka, between the period from December 2019 to June 2021 were enrolled for this study. These subjects were selected consecutively as and when they present with following inclusion and exclusion criteria.

Study design- Case control study.

Period of study- December 2019 to June 2021

Methodology: Ethical committee clearance has been taken from institutional ethical committee. 65 Patients with type 2 DM attending Outpatient department of ophthalmology fitting in inclusion criteria will be taken for study. Informed consent was taken. Patients will be distributed in 2 groups.

- Controls (Age matched normal subjects)- Group A
- Cases (Type 2 Diabetes Mellitus)- Group B

Ophthalmological examination

- Visual acuity (best corrected) using Snellen's Chart.
- Refraction
- Near vision with Times New Roman Chart.
- Slit lamp biomicroscopy examination.
- IOP with Goldman's applanation tonometry.
- Fundus examination with direct ophthalmoscope and Indirect Ophthalmoscope.
- Corneal endothelial cell count is assessed by Non-contact Topcon SP 3000 PS specular Microscope.

Inclusion criteria for cases

- Patient who will give informed consent.
- Patient with Type 2 Diabetes mellitus according to American Diabetic Association criteria 11 - A patient is grouped as diabetic who is diagnosed, or is a known case of diabetic based on his/her past history of getting treated for diabetes, self referred patients or referred from other departments of the same hospital as a part of standard treatment protocol. OR
- If the patient's blood test reports are as follows;
- Any one of the following is diagnosed as diabetic based on; Diagnostic criteria by the American Diabetic Association (ADA)¹¹
- HbA1C level more than 6.5%
- A fasting plasma glucose level of 126mg/dl or higher.
- A 2 hours plasma glucose level of 200mg/dl

or higher during a 75g oral glucose tolerance test (OGTT)

- A random plasma glucose of 200 mg/dl or higher with classic symptoms of hyperglycemia or hyperglycemic crisis.
- Cataract grading by LOCS III classification done and nucleus sclerosis grades 1-4, all cortical cataract grades and all posterior subcapsular grades included. (Fig. 1)

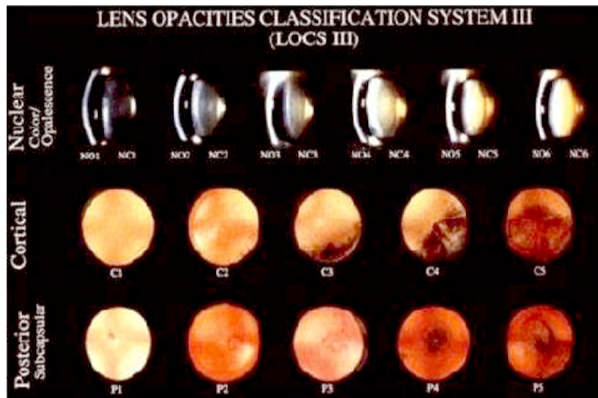


Fig. 1 : LOCS III classification

Inclusion criteria for controls

Age and sex matched healthy controls who are nondiabetic will be selected from patient attenders or patients who are coming to OPD for some other eye problems.

Exclusion criteria

- Patient not willing to give informed consent
- History of past intraocular surgeries
- Type 1 Diabetes
- Corneal diseases or dystrophies
- Contact lens users
- Active eye infections or inflammations
- Pterygium
- Glaucoma
- Posterior Staphyloma
- Keratectasia

Procedure of Specular microscopy

Corneal endothelial cell morphology and endothelial cell density is examined in all eyes by non contact specular microscope (Topcon SP 3000P). The endothelial structure is studied by measuring variety of features including endothelial

cell density, coefficient of variation and percentage of hexagonal cells. (Fig. 2)



Fig. 2: Procedure of Specular Microscopy

SAMPLE SIZE ESTIMATION

Sample size calculation was done with reference study conducted by El-agamy A9

Sample size calculated 63 in each group

RESULTS

65 patients of diabetic and 65 patients of non-diabetic group were included in the study.

Statistical methods

Data collected entered in Microsoft excel sheet, there after analysed using SPSS software version 19. Chi square test and student's "t" test used to compare corneal endothelial density and cell morphology between diabetic and non-diabetic patients. Odds ratio with 95% confidence interval calculated and p value less than 0.05 is considered as significant.

Demography and epidemiology: In the current study, mean age group of controls is 61.59 ± 5.38 and mean age group of cases is 61.79 ± 5.08 . The study included 71 (54.6%) males and 59 (45.4%) females.

AGE

In the current study, mean age group of non-diabetics is 61.59 ± 5.38 and mean age group of diabetics is 61.79 ± 5.08 . Out of 130, there were 30 patients in <65 age group, 100 patients in >65 age group. Out of 65 non-diabetics 53 (81.5%) patients were less than 65 year old & 12 patients (18.5%) fall in to more than 65 years age category and in the

diabetic group 47 patients (72.3%) were less than 65 years old & remaining 18 (27.7%) were more than 65 years old.

SEX

Among the total patients 45.4% (59) were females and 54.6% (71) were males. In non-diabetic (control) group, 13 (47.7%) are male and 34 (52.3%) are female. In diabetic group, 40 (61.5%) are male and 25 (38.5%) are female.

Descriptive Analysis of Eye

Out of 130 patients, 62 (47.7%) patient's left eye was included and 68 (52.3%) patient's right eye was included in the study. Among group A (Control), 30 (46.2%) patients left eye is included and 35 (53.8%) patients right eye is included. Among group B (Cases), 32 (49.2%) patients left eye is included and 33 (50.8%) patients right eye is included.

Endothelial cell Density

In group A the mean endothelial cell density (ECD) is 2502.75 ± 186.25 cells/mm² and in the Group B the mean ECD is 2306.08 ± 286.80 cells/mm². The mean endothelial cell density was lower in diabetic than in non-diabetic. There was significant difference in mean ECD between two groups ($P < 0.05$). (Fig. 3, Table 1)

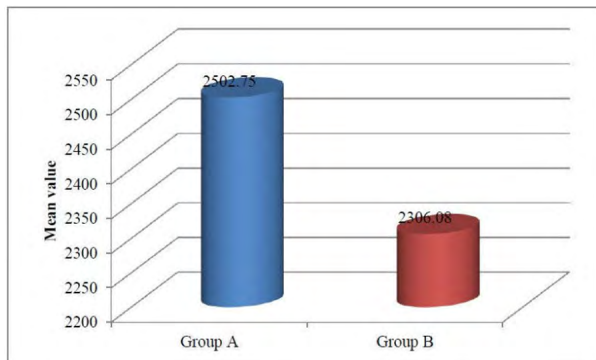


Fig. 3: Mean ECD between two groups

Coefficient of Variation

In group A the mean coefficient of variation (CV) is $36.79 \pm 3.35\%$ and in group B mean CV is $41 \pm 8.89\%$. There is statistically significant difference in mean CV between two groups were $P < 0.05$.

Hexagonal Cells(6A)

The mean hexagonal cells in group A is $45.19 \pm 3.70\%$ and in group B the mean hexagonal cells is $41.88 \pm 6.72\%$. The mean hexagonality was

lower in diabetics than in non-diabetic. There is significant difference between two groups $P < 0.05$

Random Blood Sugar (RBS)

In group A the mean RBS is 114.26 ± 15.25 and in group B the mean RBS is 141.51 ± 22.94 . There is increased RBS in diabetic group than in non-diabetic group which is statistically significant (P value < 0.001)

Group B is divided into duration of diabetes for the correlation of endothelial cell density and cell morphology.

Endothelial Cell Density (ECD) With Duration of Diabetes

The mean endothelial cell density was correlated with duration of diabetes. There was significant reduction in the mean ECD among diabetic patients with duration of diabetes > 10 years ($P < 0.05$) (Table 2, Fig. 4).

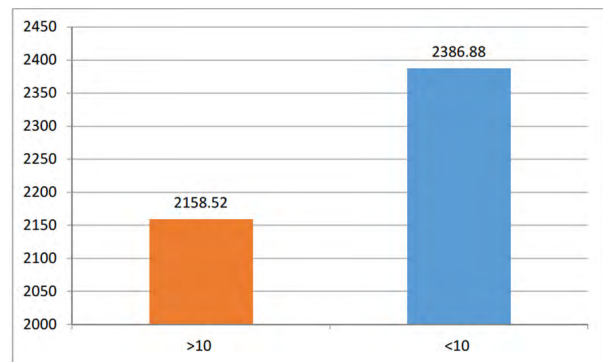


Fig. 4: Comparison of mean ECD with duration of diabetes

Coefficient of Variation (Cv) with Duration of Diabetes

Coefficient of variation was correlated with duration of diabetes. There was no significant change in the mean coefficient of variation among diabetic patients with duration of diabetes > 10 years.

Hexagonal Cells (6A) with Duration of Diabetes

Hexagonal Cells was correlated with duration of diabetes. There was very slight increase in the mean hexagonal cells among diabetic patients with duration of diabetes > 10 years.

DISCUSSION

This study entitled "Corneal endothelial cell density and cell morphology in type 2 diabetic

mellitus patients: A case control study" was conducted in the department of ophthalmology, S Nijalingappa Medical College & HSK Hospital, Bagal kotusing Topcon SP 3000PS peculiar Microscope.

In our study, we evaluated corneal endothelium & cell morphology in 130 eyes of 130 patients, with 65 patients of diabetic and 65 age matched normal subjects. Our study design is similar to other previous studies in the literature that were done to evaluate corneal endothelial cell density in diabetes.

Diabetes mellitus prevalence is rising all over the globe and is considered as a major cause of morbidity and mortality affecting young and middle age persons. The International diabetes federation (IDF) estimated that in India the diabetic subjects to be around 40.9 million and there is further set off to 69.9 million by the year 2025.¹²

Globally T2 DM approximately present 1 in every 11 adults and about 75% of patients of diabetes live in developing countries.¹³ Hyperglycemia causes toxic to cells in the body. The ocular complications of hyperglycemia are profound in retina and cornea. About 70% of diabetic patients suffer from corneal complications called as diabetic keratopathy.¹⁴

Age incidence

Lipscombe LL et al conducted a study entitled "Trends in diabetes prevalence, incidence and mortality in Ontario, Canada 1992-2005: a population based study" have found that higher prevalence rate of diabetes above 50 years or older is remained same than in those aged 20-49 years.¹⁵ In our study the mean age group is 61.79±5.08 years in Diabetic group and 61.59±5.38 years in Non diabetic group. There results of our study show similar as above mentioned.

Sex incidence

Nagaraj G et al conducted a study entitled "Corneal thickness and endothelial cell density in diabetic and non-diabetic patients: a hospital based comparative study" have found that among diabetics the female ratio was more than male and in non-diabetic males were more than females.¹⁶ In our study the female prevalence was higher than males in non-diabetic groups and male prevalence was higher than females in diabetic groups. In diabetic group, females were 38.5% (25) and males were 61.5% (40). In non-diabetic

group, females were 52.3%(34) and males were 47.7%(31).

Endothelial cell density

K.I noueetal conducted study entitled "The corneal endothelium and thickness in type 2 diabetes mellitus" investigated the corneal morphology in diabetic and non-diabetic and found significant decrease in endothelial cell density. 17 Sudhir et al conducted the study entitled "Changes in the corneal endothelial cell density and morphology in patients with type 2 diabetes mellitus: a population based study and thus concluded that, subjects with type 2 diabetes mellitus had lower endothelial cell counts in comparison with non-diabetic controls. 4 Our study was similar to above mentioned studies, the endothelial cell density is decreased in the diabetic group when compared with the non-diabetics.

Choo MM et al conducted the study entitled "Corneal changes in type II diabetes mellitus in Malaysia" concluded that endothelial cell density was significantly lower in diabetic group (2541.6±516.4 cells/mm²) when compared to non-diabetic (2660.1±515.5 cells/mm²).⁷ El-agamy A et al conducted a study entitled "Corneal endothelium and central corneal thickness changes in Type 2 Diabetes Mellitus" concluded that ECD was significantly lower in Diabetic cornea group (2,491.98±261.08 cell/mm²) than in the control group (2,629.68±293.45 cell/mm²).⁹ Nagaraj G et al conducted study entitled "Corneal thickness and endothelial cell density in diabetic and non-diabetic patients: a hospital based comparative study" and found that the endothelial cell density was significantly lower (2438.73±250.23 cells/mm²) in the diabetic group when compared with non-diabetics (2599.88±168.16 cells/mm²).¹⁶ These studies were similar to our study were the mean endothelial cell density is significantly lower compared to non-diabetics.

Elsobky et al conducted study entitled "Corneal endothelial and central corneal thickness changes in patients with type 2 diabetes mellitus" found that the mean endothelial cell count was significantly decreased in the diabetic group. In this study, the diabetic group was sub divided into disease of duration and stage of retinopathy and they found that there was statistically significant difference in mean ECD between patients having diabetes for <10 years duration and those having diabetes >10 years duration.¹⁸

This study was similar to our study endothelial cell density was decreased and found significant in diabetic group of duration >10 years compared to those < 10 years of duration.

Correlation of Endothelial cell density with diabetic duration

JS Lee et al conducted study entitled "Differences in corneal thickness and corneal endothelium related to duration in diabetes" observed that the ECD was lower for diabetes of over 10 years duration than for diabetes of under 10 years.¹⁹ This study was similar to our study in which the mean ECD was significantly lower in patients with duration of diabetes >10 years.

Stella Brigg setal conducted study entitled "Manifestations of Type 2 Diabetes in Corneal endothelial cell density, Corneal thickness and Intraocular pressure" demonstrates that there was significant difference in mean ECD of diabetic patients with duration of diabetes >10 years when compared with duration of diabetes <10 years.³ Our study is similar to this study as there is significant difference in mean ECD value in diabetes with diabetic duration >10 years.

CONCLUSION

This study titled "Corneal endothelial cell density and cell morphology in type 2 diabetes mellitus patients: A case control study." was conducted in Department of Ophthalmology, SNMC & HSK Hospital, Karnataka from December 2019 to June 2021.

130 patients were included in the study, 65 patients who are diagnosed with type 2 DM and 65 patients were age matched controls.

In this study, age of the patient ranged from 50 to 80 years, with mean age being 61.79 ± 5.08 years in Diabetic group and 61.59 ± 5.38 years in Non diabetic group. In this study, the mean endothelial cell density in diabetic group 2306.08 ± 286.80 cells/mm² and in non-diabetic group 2502.75 ± 186.25 cells/mm².

In patients with diabetes has lower endothelial cell density and found to be significant decrease in the mean ECD. Coefficient of variation is higher in diabetic group when compared to non-diabetic control group. The hexagonality percentage is lower in diabetics than in non-diabetics.

Hexagonality percentage is lower in diabetic group comparing to non-diabetic and the reduction

is significant in diabetic group which is comparable to the most of studies earlier. In this study the duration of disease is correlated with endothelial cell density. There is significant decreased ECD in diabetic duration >10 years and found positive correlation with duration of diabetes with endothelial cell density and discussed earlier with some of the studies. We observed that the diabetic corneas are at higher risk of damage to the corneal endothelium.

Lower endothelial cell count in diabetes is due to glycaemic stress and the age also can be a founding factor for decrease in the endothelial cell density along with diabetes mellitus. This could be contributed by any difference in inclusion or exclusion criteria with duration of diabetes, glycaemic control, and also severity of disease. The endothelial cell density was decreased in diabetic group when compared to non-diabetic group. 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. So specular microscopy can thus be used as a tool for studying the corneas of diabetic patients and it is imperative that corneal specular microscopy is to be done routinely preoperatively for diabetic patient even if cornea appears to be healthy.

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