RESEARCH PAPER

To Compare Endothelial Cell Density, Cell Size, And Polymegathism In Diabetic And Non-Diabetic Populations

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ABSTRACT

Purpose:

To compare corneal endothelial cell density, cell size variation, and polymegathism between diabetic and non-diabetic populations using specular microscopy.

Methods:

This hospital-based cross-sectional observational study included 108 participants, comprising 54 non-diabetic individuals and 54 patients with Type 2 Diabetes Mellitus aged above 30 years. Subjects were selected based on clinical history and divided into two groups. All participants underwent comprehensive ophthalmic examination including visual acuity assessment, intraocular pressure measurement, and colour vision testing. Corneal endothelial analysis was performed using non-contact specular microscopy. The parameters evaluated included endothelial cell density (ECD), coefficient of variation (CV), mean cell area, and hexagonality (HEX). Additional parameters such as corneal thickness and curvature were also recorded. Data were analyzed using analytic statistics. Comparative analysis between groups was performed using independent t-test or Mann–Whitney U test. Correlation between duration of diabetes and endothelial parameters was assessed. A p-value < 0.05 was considered statistically significant.

Results:

The mean endothelial cell density (ECD) in the study population was approximately 2625.4 ± 520.3 cells/mm². On subgroup analysis, diabetic patients showed a relatively lower mean ECD compared to non-diabetic individuals. The mean coefficient of variation (CV) was 34.7 ± 7.1%, indicating increased variability in endothelial cell size, with higher values observed in the diabetic group suggesting greater polymegathism. The mean hexagonality (HEX) was 63.2 ± 9.4%, with a reduction noted among diabetic subjects, reflecting increased pleomorphism. The mean central corneal thickness (CCT) was 518 ± 35 µm. Additionally, a trend of decreasing endothelial cell density and hexagonality, along with increasing coefficient of variation, was observed with longer duration of diabetes. Overall, endothelial morphological changes were more pronounced in diabetic patients compared to non-diabetic controls.

Conclusion:

Diabetes mellitus causes changes in corneal endothelial morphology, including reduced cell density, increased polymegathism, and decreased hexagonality. Specular microscopy is useful for early detection and monitoring of these changes.

Keywords: Diabetes mellitus, corneal endothelium, endothelial cell density, polymegathism, specular microscopy.

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both. It is a major global health concern because of its increasing prevalence and association with various systemic complications. Among the organs affected, the eye is particularly vulnerable, with well-known complications such as diabetic retinopathy, cataract, and glaucoma.[1–3] The cornea is a transparent, avascular structure that plays an essential role in maintaining the optical clarity and refractive power of the eye. The corneal endothelium, a single layer of hexagonal cells located on the posterior surface, is responsible for maintaining corneal transparency by regulating stromal hydration through its barrier and pump function.[4–6]Corneal endothelial cells have very limited regenerative capacity. Therefore, when cell loss occurs due to aging or disease, the remaining cells enlarge and change shape to compensate. These morphological changes include variation in cell size (polymegathism) and variation in cell shape (pleomorphism), which can affect corneal function if significant.[5,6] Evaluation of corneal endothelial morphology is commonly performed using specular microscopy. Important parameters include endothelial cell density (ECD), coefficient of variation (CV) representing polymegathism, and percentage of hexagonal cells (HEX) indicating pleomorphism. These parameters provide valuable information regarding the structural and functional status of the corneal endothelium.[7–9] In normal individuals, endothelial cell density decreases gradually with age. However, several systemic and ocular conditions can accelerate endothelial cell loss. Diabetes mellitus is one of the major systemic diseases associated with corneal endothelial alterations, leading to decreased cell density and increased variability in cell size and shape.[10–15]

Chronic hyperglycemia contributes to corneal endothelial damage through multiple mechanisms. Formation of advanced glycation end products (AGEs), activation of the polyol pathway with sorbitol accumulation, oxidative stress, and reduced Na^+/K^+-ATPase activity all play a role in endothelial dysfunction in diabetic patients.[13–18] In addition, diabetic neuropathy affects corneal innervation, leading to reduced corneal sensitivity and altered corneal metabolism. These changes may indirectly influence endothelial cell function and contribute to corneal abnormalities observed in diabetic patients.[19–21]

Fig 1 : Specular microscopy[34]

Specular microscopy is a non-invasive and reliable method for assessing corneal endothelial morphology. It allows quantitative evaluation of endothelial parameters and helps detect early subclinical changes in diabetic patients, even before clinical manifestations appear.[22–24] Several studies have demonstrated significant differences in corneal endothelial parameters between diabetic and non-diabetic individuals. Diabetic patients commonly show reduced endothelial cell density, increased polymegathism, and decreased hexagonality. Furthermore, these changes have been shown to correlate with the duration of diabetes and level of glycemic control.[25-30]

These endothelial changes are clinically important, especially in patients undergoing intraocular surgeries such as cataract extraction. Diabetic patients may have a higher risk of endothelial cell loss and postoperative corneal edema due to pre-existing endothelial compromise. Therefore, preoperative evaluation of endothelial parameters is essential in such patients.[27–31]

Recent studies (2020–2025) have further confirmed that diabetes mellitus causes progressive corneal endothelial alterations and that these changes may serve as early indicators of systemic micro vascular damage. Advances in imaging techniques have improved the understanding of diabetic corneal changes and highlighted the importance of routine corneal evaluation in diabetic patients.[32–33]

Despite extensive research, variations in findings exist due to differences in study populations and methodologies. Hence, further studies are required to compare corneal endothelial parameters between diabetic and non-diabetic individuals. Therefore, the present study aims to compare endothelial cell density, cell size variation, and polymegathism in diabetic and non-diabetic populations using specular microscopy.

Material and Methods

The present hospital-based cross-sectional observational study was conducted at the Department of Optometry in collaboration with the Department of Ophthalmology at NIMS Hospital, NIMS University Rajasthan, Jaipur, India, over a period of three months. written informed consent was taken from all participants prior to inclusion in the study. A total of 108 participants were included in the study, comprising 54 patients diagnosed with Type 2 Diabetes Mellitus and 54 age-matched non-diabetic individuals who served as controls. Both eyes of each participant were evaluated. Participants with a history of contact lens wear, ocular trauma, previous ocular surgery, intraocular inflammation, corneal pathology, or use of topical ocular medications were excluded from the study. A detailed medical and ocular history was obtained from all participants. Information regarding age, gender, duration of diabetes, and associated systemic conditions such as hypertension was recorded. All participants underwent a comprehensive ophthalmic examination, including visual acuity assessment using Snellen chart, slit-lamp biomicroscopy of the anterior segment, measurement of intraocular pressure using non-contact tonometry, and fundus evaluation. Corneal endothelial morphology was assessed using a non-contact specular microscope (NIDEK CEM-530, Japan). Participants were instructed to fixate on the internal target while images of the central cornea were captured. Multiple images were obtained for each subject, and the best-quality image with clearly visible endothelial cell borders was selected for analysis. The following endothelial parameters were evaluated using the inbuilt software of the specular microscope:

- **Endothelial Cell Density (ECD):** number of cells per mm^2
- **Coefficient of Variation (CV):** indicating polymegathism
- **Percentage of Hexagonal Cells (HEX):** indicating pleomorphism
- **Central Corneal Thickness (CCT):** measured in micrometers

Approximately 100 contiguous endothelial cells were analyzed in each image to ensure accuracy and reproducibility. The primary objective of the study was to compare endothelial cell density, cell size variation, and polymegathism between diabetic and non-diabetic populations. Statistical analysis was performed using appropriate tests, and a p-value < 0.05 was considered statistically significant.

Statistical Analysis

All data were entered and analyzed using statistical software. Quantitative variables were expressed as **mean ± standard deviation**, while categorical variables were presented as **frequency and percentage**. Comparison between diabetic and non-diabetic groups was performed using the **independent Student's t-test** for continuous variables. The **chi-square test** was used to analyze categorical variables. Correlation analysis was also performed to evaluate the relationship between endothelial parameters and diabetic status.

A **p-value < 0.05** was considered statistically significant.

RESULTS

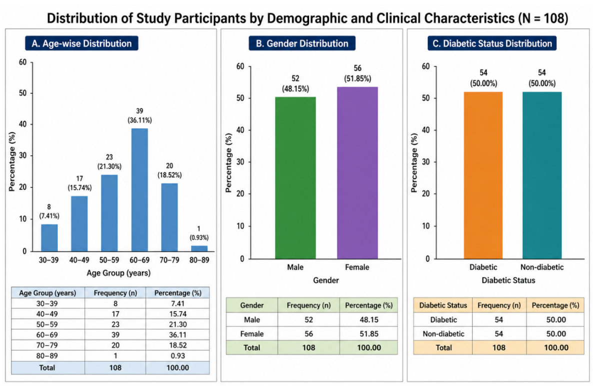
In the present study, a total of **108 participants** were evaluated to analyze corneal endothelial morphology using non-contact specular microscopy. The study population comprised **54 patients with diabetes mellitus and 54 age-matched non-diabetic controls**. All participants underwent a comprehensive ophthalmological evaluation, including visual acuity assessment and intraocular pressure measurement. No significant abnormalities were detected during routine clinical examination, and all subjects had clear corneas suitable for endothelial analysis.

Demographic Characteristics

The demographic profile of the study population is summarized in Graph 1. The age of participants ranged from **30 to 89 years**, with the majority belonging to the **60–69 years age group (36.11%)**, followed by the 50–59 years (21.30%) and 70–79 years (18.52%) categories. Younger age groups constituted a relatively smaller proportion, with 7.41% in the 30–39 years category and 15.74% in the 40–49 years group. Only one participant (0.93%) was observed in the 80–89 years age group.

Gender distribution showed a nearly equal representation, with **56 females (51.85%) and 52 males (48.15%)**, indicating a slight female predominance. The study design ensured equal representation of diabetic and non-diabetic individuals, with **54 participants (50.00%) in each group**, allowing for a balanced comparative analysis.

Graph 1. Demographic Characteristics of Study Population (n = 108)

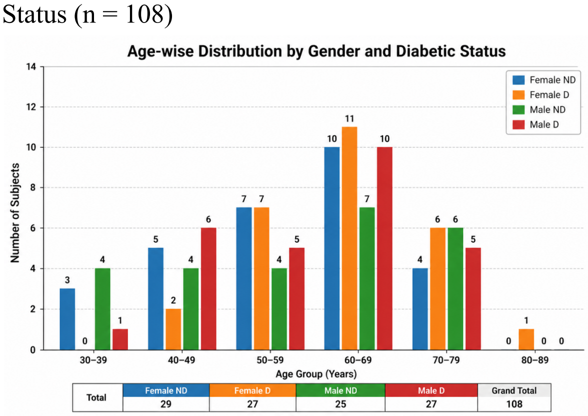


Age-wise Distribution by Gender and Diabetic Status

The age-wise distribution of participants stratified by gender and diabetic status is presented in Graph 2. The highest concentration of participants was observed in the **60–69 years age group (n = 38)**, which included both diabetic and non-diabetic individuals across genders. Among females, 29 were non-diabetic and 27 were diabetic, while among males, 25 were non-diabetic and 27 were diabetic.

The 50–59 years age group accounted for 23 participants, followed by 21 participants in the 70–79 years group. The distribution pattern remained consistent across gender and diabetic status, indicating a well-balanced sample population suitable for comparative analysis.

Graph 2. Age-wise Distribution by Gender and Diabetic Status (n = 108)



Hypertension Status

The distribution of systemic hypertension among study participants is summarized in Table 1. A total of **24 participants (22.22%)** were identified as hypertensive, whereas the majority **84 participants (77.78%)** were non-hypertensive.

This finding indicates that most participants did not have associated systemic hypertension, thereby reducing potential confounding effects on corneal endothelial parameters.

Table 1. Distribution of Hypertension Status (n = 108)

Hypertension Status	**Frequency (n)**	**Percentage (%)**
Hypertensive	24	22.22
Non-hypertensive	84	77.78
Total	108	100

Corneal Endothelial Cell Density (ECD) Status

The distribution of endothelial cell density (ECD) status among diabetic and non-diabetic participants is presented in Table 2. The majority of participants exhibited normal endothelial cell density. However, a higher proportion of abnormal ECD was observed among diabetic individuals.

Among diabetic patients, **47 (87.04%)** had normal ECD, while **7 (12.96%)** demonstrated reduced endothelial cell density. In contrast, among non-diabetic participants, **53 (98.15%)** had normal ECD and only **1 (1.85%)** showed abnormal findings.

Table 2. Endothelial Cell Density (ECD) Status According to Diabetic Status

ECD Status	**Diabetic (n = 54)**	**Non-diabetic (n = 54)**	**Total**
Normal	47 (87.04%)	53 (98.15%)	100 (92.59%)
Abnormal	7 (12.96%)	1 (1.85%)	8 (7.41%)
Total	54	54	108

Comparison of Corneal Endothelial Parameters

The quantitative comparison of corneal endothelial parameters between diabetic and non-diabetic participants is presented in Table 3. The parameters evaluated included endothelial cell density (ECD), coefficient of variation (CV), percentage of hexagonal cells (HEX), and central corneal thickness (CCT).

Table 3. Comparison of Corneal Endothelial Parameters Between Diabetic and Non-Diabetic Groups

Parameter	**Diabetic (n = 54) Mean ± SD**	**Non-diabetic (n = 54) Mean ± SD**	**p-value**
Endothelial Cell Density (cells/mm²)	2500.17 ± 462.52	2734.15 ± 299.57	0.0028*
Coefficient of Variation (CV %)	33.4 ± 6.2	31.8 ± 5.4	>0.05
Hexagonality (HEX %)	64.8 ± 8.7	66.9 ± 7.9	>0.05
Central Corneal Thickness (µm)	528 ± 32	521 ± 28	>0.05

*Statistically significant ($p < 0.05$)

Interpretation of Corneal Endothelial Parameters

The analysis demonstrated a **statistically significant reduction in endothelial cell density (ECD)** in diabetic patients compared to non-diabetic controls ($p = 0.0028$). The mean ECD in diabetic individuals was **2500.17 ± 462.52 cells/mm²**, whereas in non-diabetic participants it was **2734.15 ± 299.57 cells/mm²**, indicating an approximate **8–9% reduction** in endothelial cell density among diabetics.

Although the **coefficient of variation (CV)** was slightly higher in diabetic patients (33.4 ± 6.2%) compared to non-diabetics (31.8 ± 5.4%), the difference was not statistically significant ($p > 0.05$). This suggests a trend toward increased polymegathism in diabetic individuals.

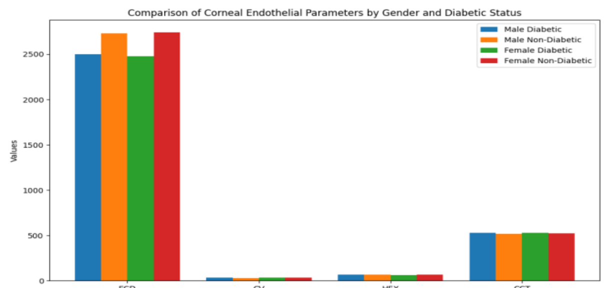
Similarly, the **percentage of hexagonal cells (HEX)** was marginally lower in diabetics (64.8 ± 8.7%) than in non-diabetics (66.9 ± 7.9%), indicating a mild degree of pleomorphism; however, this difference was also not statistically significant.

The **central corneal thickness (CCT)** was slightly higher in diabetic patients (528 ± 32 µm) compared to non-diabetic individuals (521 ± 28 µm), suggesting a tendency toward increased corneal thickness, although this finding did not reach statistical significance.

Overall Interpretation

These findings indicate that **diabetes mellitus is primarily associated with a significant reduction in endothelial cell density**, while other morphological parameters such as CV, HEX, and CCT show subtle, non-significant changes. The observed reduction in ECD reflects early endothelial dysfunction and reduced cellular reserve in diabetic patients.

Graph 3: Comparison of corneal endothelial parameters by gender and diabetic status



Scatter Plot Analysis

Scatter plot analysis was performed to evaluate the relationship between diabetic status and corneal endothelial parameters, including endothelial cell density (ECD), coefficient of variation (CV), hexagonality (HEX), and central corneal thickness (CCT).

The scatter plot illustrating the relationship between **endothelial cell density (ECD) and diabetic status** demonstrated a **negative correlation**, indicating that diabetic individuals tend to have lower endothelial cell density compared to non-diabetic participants. This trend reflects endothelial cell loss associated with diabetes mellitus.

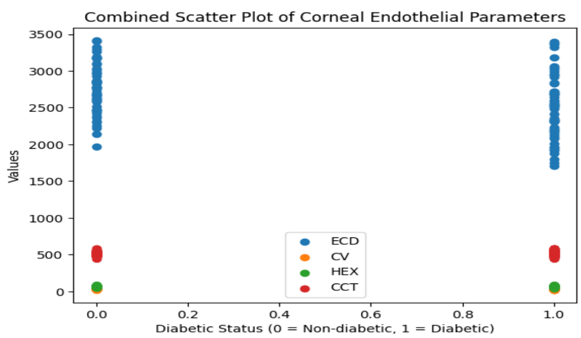
Similarly, the scatter plot for **coefficient of variation (CV)** showed a **slight positive association with diabetic status**, suggesting increased variability in endothelial cell size (polymegathism) among diabetic patients. However, the dispersion of data points indicates that this relationship was weak and not statistically significant.

The analysis of **hexagonality (HEX)** revealed a **mild negative trend**, with diabetic individuals showing a tendency toward reduced percentage of hexagonal cells.

This suggests early pleomorphic changes in the corneal endothelium, although the correlation was weak.

In the case of **central corneal thickness (CCT)**, the scatter plot demonstrated a **slight positive relationship with diabetic status**, indicating a tendency toward increased corneal thickness in diabetic patients. However, the spread of values overlapped considerably between groups, suggesting no strong association.

Overall, the scatter plot analysis highlights that **endothelial cell density is the most significantly affected parameter in diabetic patients**, showing a clear downward trend. In contrast, CV, HEX, and CCT exhibited only mild variations with substantial overlap between diabetic and non-diabetic groups, indicating less pronounced changes.



Conclusion of Scatter Analysis;;

- **ECD:** Moderate negative correlation (most significant finding)
- **CV:** Weak positive trend (polymegathism)
- **HEX:** Weak negative trend (pleomorphism)
- **CCT:** Mild positive trend (increased thickness)

DISCUSSION

The present study evaluated corneal endothelial morphology in diabetic and non-diabetic individuals using non-contact specular microscopy. The results demonstrated a statistically significant reduction in endothelial cell density (ECD) in diabetic patients, along with mild, non-significant changes in coefficient of variation (CV), hexagonality (HEX), and central corneal thickness (CCT).

The significant decrease in ECD observed in diabetic subjects is consistent with previous studies. **Schultz et al.,**(7) first reported endothelial cell loss in diabetes, attributing it to chronic metabolic stress. Similar findings were reported by **Roszkowska et al.,**,(8) who demonstrated reduced endothelial cell density in diabetic populations. **Storr-Paulsen et al.,** (9)further confirmed these observations, suggesting that diabetes accelerates endothelial cell loss beyond normal aging.

In the present study, CCT was slightly higher in diabetic individuals, although not statistically significant. This finding is in agreement with studies by **Urban et al.,** (15)and **Su et al.,**,(13) who reported increased corneal thickness in diabetic patients due to endothelial pump dysfunction.

The observed increase in CV in diabetic patients indicates a trend toward polymegathism, reflecting variability in endothelial cell size. Although not statistically significant, this finding is comparable to results reported by **Lee et al.,** (10)and **Choo et al.,**,(16) who found increased polymegathism in diabetic corneas. Similarly, the slight reduction in HEX suggests early pleomorphic changes, which has also been reported by **Sudhir et al.,** (14)and **Shenoy et al.,**.(17)

The pathophysiological mechanisms underlying these changes include chronic hyperglycemia-induced oxidative stress, accumulation of advanced glycation end products (AGEs), and activation of the polyol pathway. These factors impair endothelial cell metabolism and function. As described by **Morikubo et al.,**,(12) reduced Na^+/K^+-ATPase activity compromises endothelial pump function, leading to corneal edema. Additionally, **Rosenberg et al.,B**(19) highlighted that diabetic neuropathy may affect corneal innervation, indirectly influencing endothelial health.

The present study also suggests that endothelial alterations may progress with increasing duration of diabetes. This observation is supported by **Rashmi et al.,** (26)and **Gendy et al.,**,(22) who reported a correlation between disease duration and endothelial damage. A recent meta-analysis by **Sharifi et al.,** (23)further confirmed significant alterations in endothelial parameters in diabetic patients.

Clinically, these findings are important because corneal endothelial cells have limited regenerative capacity, as emphasized by **Bourne**(4). Reduced endothelial reserve increases the risk of corneal edema and postoperative complications. Studies such as **Yadav et al.,**(27) have demonstrated increased endothelial cell loss in diabetic patients following cataract surgery, highlighting the need for preoperative endothelial evaluation.

The limitations of this study include its cross-sectional design and relatively small sample size. Additionally, glycemic control parameters such as HbA1c were not extensively analyzed. Future longitudinal studies with larger populations are recommended.

In conclusion, this study supports existing evidence that diabetes mellitus leads to early and progressive corneal endothelial alterations, particularly reduced ECD, with mild changes in CV, HEX, and CCT.

CONCLUSION

The present study concludes that diabetes mellitus is associated with mild alterations in corneal endothelial morphology, including reduced endothelial cell density, increased coefficient of variation, and slight changes in hexagonality. A mild increase in central corneal thickness was also observed.

These findings suggest that diabetes can cause early endothelial changes, even without clinical symptoms, and that duration of diabetes may influence these alterations.

Regular corneal endothelial evaluation is important in diabetic patients, especially before intraocular procedures. Further studies with larger samples and long-term follow-up are recommended.

Competing Interests

The author declares that there are no competing interests regarding the publication of this study.

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