Original Article

# Evaluation and correlation of corneal endothelium parameters with the severity of primary glaucoma

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**Purpose:** To evaluate and correlate corneal endothelium parameters with the severity of primary glaucoma. **Methods:** This prospective case-control study was conducted on 150 eyes of 80 newly diagnosed primary open-angle glaucoma (POAG), chronic primary angle-closure glaucoma (cPACG), and normal-tension glaucoma (NTG) patients in a tertiary care center. Endothelial parameters including endothelial cell count (ECC), percentage of hexagonal cells, and coefficient of variation of cell size were analyzed. Glaucoma cases were further sub-grouped into early, moderate, and severe glaucoma and compared for endothelial parameters. Chi-square, Fischer's exact test, independent sample t-test, and analysis of variance were performed using IBM® SPSS® Statistics version 28. **Results:** The ECC was markedly reduced in cases (2281 cells/mm$^2$) versus controls (2611 cells/mm$^2$) ($P < 0.001$). The POAG (2251 cells/mm$^2$) and cPACG (2287 cells/mm$^2$) eyes had significantly a lower ECC compared to healthy controls ($P < 0.001$), whereas NTG eyes had a lower mean ECC (2538 cells/mm$^2$), but it was not statistically significant ($P > 0.05$) when compared with controls. The ECC decreased with an increase in severity as patients with early glaucoma had an ECC of 2284 cells/mm$^2$, moderate 2261 cells/mm$^2$, and severe 2086 cells/mm$^2$, and the difference was statistically significant. **Conclusion:** A significant decrease in corneal ECC was observed in POAG and cPACG patients when compared with healthy controls of the same age group. Mechanical damage following an elevated intra-ocular pressure for a longer duration may be attributed to morphological and consequent functional damage to endothelial cells.

**Key words:** Chronic primary angle-closure glaucoma, endothelial cell density, intra-ocular pressure, normal-tension glaucoma, primary open-angle glaucoma

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The corneal endothelium is a single layer of hexagonal cells having an important role in regulating stromal hydration and maintaining corneal clarity through its pump mechanism. These cells have limited regenerative capacity; thus, damage to them is irreversible. At an early stage of endothelial cell damage, functional damage is compensated by enlargement and migration of neighboring cells, but continuous damage of endothelial cell loss leads to their de-compensation, resulting in corneal swelling and loss of clarity.[1] There is increasing evidence that glaucoma is one of the many pathologies which damage corneal endothelial cells.

Glaucoma refers to a group of diseases characterized by degeneration of retinal ganglion cells, followed by distinct optic nerve head features and correlated visual field changes.[2-4] Unlike the epithelium of the cornea, endothelial cells lack self-renewal ability, making it essential to preserve these cells by understanding the risk factors associated with endothelial loss in glaucoma. Endothelial cell loss has been reported in primary open-angle glaucoma (POAG), primary angle-closure glaucoma (PACG), and pseudo-exfoliation glaucoma.[2-4] Endothelial cell loss in glaucoma has been ascribed to various factors including direct trauma because of an elevated intra-ocular pressure (IOP), long-term anti-glaucoma medication toxicity, and following glaucoma filtration surgery more so after aqueous shunt implantation.[5]

In this study, we aimed to observe the corneal endothelial changes in newly diagnosed patients of POAG, closed-angle glaucoma, and normal-tension glaucoma (NTG) to correlate it with their mean IOP by comparing them with age and sex-matched controls and with the severity of glaucoma by comparing endothelial cell changes among newly diagnosed glaucoma patients.

## Methods

After taking permission from the institutional ethical committee, the present prospective observational study was conducted in a Regional Institute of Ophthalmology, in Northern India, between January 1, 2020 and December 31, 2020. Diabetic patients, glaucoma patients on treatment, patients with secondary glaucoma, and patients with a history of intra-ocular surgery, ocular trauma, or any other ocular diseases were

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excluded from the study. Out of 193 consecutive newly diagnosed patients of glaucoma, 80 patients (150 eyes) who fulfilled the inclusion criteria and equal age and sex-matched controls were enrolled in the study after they gave written informed consent in their vernacular language following the declaration of Helsinki.

A total of 150 eyes (80 patients) were separated into three sub-groups: POAG, chronic primary angle-closure glaucoma (cPACG), and NTG.

Detailed history and comprehensive examination including best-corrected visual acuity, slit-lamp biomicroscopy, pachymetry, gonioscopy with a three-mirror Goldmann Gonio lens, intra-ocular pressure (IOP) by applanation tonometry, indirect dilated slit-lamp biomicroscopic optic disc assessment with a +90 D Lens, and visual field analysis using a Humphrey field analyzer of each patient were documented. Glaucoma patients were further sub-divided into early, moderate, and severe depending on their mean deviation on perimetry. Patients with a mean deviation of -6D were graded as early, those with a mean deviation less than -12D were graded as moderate, and those with a mean deviation greater than -12D were graded as severe.

Detailed specular microscopy was performed to examine corneal parameters including endothelial cell count (ECC), coefficient of variation (CV) of cell size, and percentage of hexagonal cells using a specular microscope (TOPCON SP-3000P).

### Statistical analysis

Group differences across categorical variables were analyzed using the Chi-square or Fischer's exact test. Differences across the cases and controls groups were assessed using the independent sample t-test for non-parametric variables. Group differences across the three groups in the continuous variables were analyzed using the analysis of variance or the Kruskal–Wallis test. All data were analyzed by using IBM® SPSS® Statistics version 28.

## Results

The mean age of 80 newly diagnosed glaucoma cases and 80 controls was 58.05 years and 58.49 years, respectively [Table 1]. Out of 80 cases, 37 were males and 42 were females. The cases were divided into three sub-groups based on the type of glaucoma diagnosed, namely, POAG, cPACG, and NTG. The endothelial parameters including endothelial cell density, coefficient of variation of cell size, and percentage of hexagonality of endothelial cells were measured by specular microscopy, and their mean was compared between cases and controls. The mean ECC in cases was significantly lower than that of controls (2281 cells/mm$^2$ vs 2611 cells/mm$^2$, $P < 0.05$), whereas the mean CV was significantly higher (35.70) in cases

**Table 1: Demographic profile of cases and controls**

| | Controls | Cases | *P* |
|---|---|---|---|
| Males | 42 | 37 | >0.05 |
| Females | 38 | 42 | >0.05 |
| Total | 80 | 80 | |
| Mean Age (in years) | 58.49 | 58.05 | >0.05 |

**Table 2: Comparison of IOP and corneal endothelial parameters of controls and cases**

| | Controls | Cases | *P* |
|---|---|---|---|
| Mean IOP mmHg | 15.13 | 27.51 | <0.05 |
| Mean ECC (cells/mm$^2$) | 2611 | 2281 | <0.05 |
| Mean CV of cell size | 27.06 | 35.70 | <0.05 |
| Mean of the percentage of hexagonal cells (%) | 68.68% | 48.40% | <0.05 |

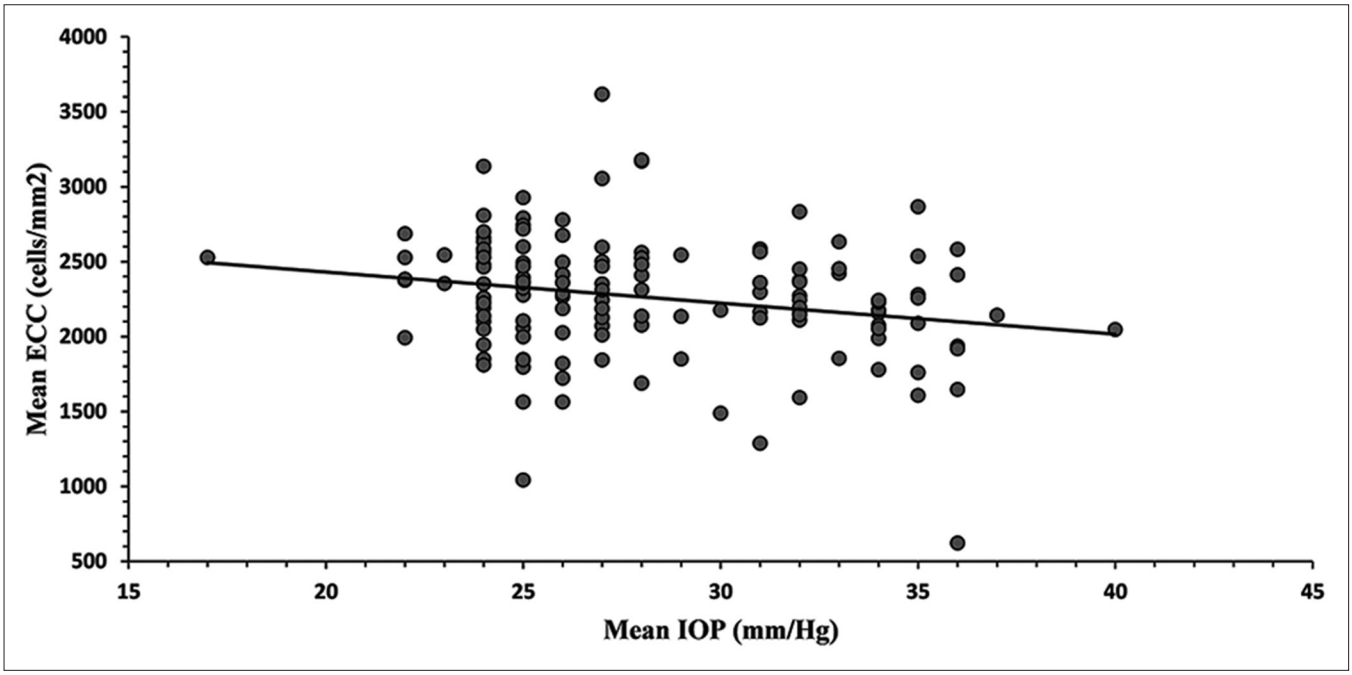


**Figure 1:** Negative linear correlation between corneal ECC and IOP

**Table 3: Comparison of corneal endothelial parameters between controls and sub-groups of glaucoma**

| Type of glaucoma | Number | Mean Endothelial cell count (cells/mm²) | Mean Coefficient of variation of cell size (CV) | Mean of Percentage of hexagonal cells (Hx %) |
|---|---|---|---|---|
| Controls | 80 | 2611 | 27.06 | 68.68% |
| POAG | 53 | 2251.19 | 36.63 | 44.99% |
| cPACG | 22 | 2287 | 35.29 | 52.69% |
| NTG | 5 | 2538.29 | 28.9 | 66.05% |
| *P* (ANOVA) | - | <0.5 | <0.05 | <0.05 |

*ANOVA: Analysis of variance test

**Table 4: Comparison of corneal endothelial parameters with severity of glaucoma**

| Endothelium parameters | Early Glaucoma (*n*=52) | Moderate Glaucoma (*n*=38) | Severe Glaucoma (*n*=6) | *P* (ANOVA) |
|---|---|---|---|---|
| Mean ECC (cells/mm²) | 2284±370 | 2261±243 | 2086±423 | < 0.001 |
| Mean CV of cell size | 29±5.6 | 36±6.4 | 42±8.2 | <0.001 |
| Mean of percentage of hexagonal cells (Hx %) | 58.7±9.6 | 44.42±14.08 | 40.18±14.03 | <0.001 |

*ANOVA: Analysis of variance test

when compared to controls (35.70 vs 27.06, $P < 0.05$). The mean percentage of hexagonal cells in cases and controls was 48.40% and 68.68%, respectively [Table 2]. The mean IOP of cases was 27.51 mm of Hg. It was significantly higher than that of controls (15.13 mmHg). A direct association of a high mean IOP and ECC was observed. With increasing IOP in cases, a decrease in the endothelial count was noticed [Fig. 1].

When endothelial parameters were compared among the sub-groups of types of glaucomas, POAG patients had the least ECC (2251 cells/mm²) and hexagonality (44.99%) and the maximum CV (36.63) of endothelial cells, whereas patients with NTG had a maximum number of endothelial cells (2538 cells/mm²) and hexagonality (67.5%) and the least coefficient of variation (25.5) of endothelial cells ($P < 0.05$) [Table 3]. The endothelial cell parameters were correlated with the severity of glaucoma, and the patients with severe glaucoma had the lowest mean ECC (2086 cells/mm²) and hexagonality (40%) and the highest CV (42) as compared to mild and moderate glaucoma [Table 4].

## Discussion

Out of 80 freshly diagnosed glaucoma patients enrolled in our present study, 53 (66.25%) patients had POAG, 22 (27.5%) had cPACG, and 5 (6.25%) had NTG. It was in accordance with the study performed by Nangia V *et al*.[6] which reports POAG as the major type of glaucoma. However, a higher prevalence of primary ACG has also been reported in a study performed by Palimkar A *et al*.[7] Our prospective case-control study revealed a 12.6% reduction in mean ECC in glaucoma cases as compared to age-matched healthy controls.

Gagnon and colleagues compared corneal ECC in different types of glaucoma and found ECC to be lower in glaucoma patients than in controls.[8] Stroligo MN in their study also concluded a lower ECC in glaucomatous eyes compared to non-glaucomatous eyes.[9]

Further, we observed that POAG (2251 cells/mm²) and cPACG (2287 cells/mm²) eyes had a significantly lower ECC compared to healthy controls ($P < 0.001$), whereas NTG eyes had a lower mean ECC (2538 cells/mm²), but it was not statistically significant ($P > 0.05$) when compared with controls. It was similar to the observations in a study conducted by Bhomaj *et al*.,[10] who revealed a significantly lower ECC in POAG patients as compared to controls. However, in a study performed by Luo, Li J, and Jin Y, the endothelium density and morphology of POAG and non-acute PACG were as similar as those of normal persons.[11]

Sung Woo Cho *et al*.[12] also observed that there is no significant reduction in corneal endothelial cell density in NTG patients compared to the normal group but a 13% reduction in ECC of POAG patients in comparison to the normal group.

The mean IOP in our study group was significantly higher as compared to healthy controls, suggesting that a high IOP may be the contributing factor for reduction in ECC. Gagnon *et al*.[13] also reported similar observations and explained that lower cell counts in patients with glaucoma are because of either direct compression of the corneal endothelium by raised IOP or congenital alteration of both the corneal endothelial cell layer and the trabecular meshwork in patients with glaucoma or glaucoma medication toxicity.

It was also confirmed by a study performed by Melamed *et al*.,[14] who showed that experimental induction of ocular hypertension in rabbits led to a decreased corneal endothelial density. They interpreted that injury to the active pump mechanism following morphological damage was responsible for resultant corneal edema. They interpreted that a high IOP affected the cornea first by direct mechanical trauma to endothelial cells and later with a persistent high IOP by injury to the active metabolic pump mechanism which keeps the cornea de-hydrated.

On contrary to their study where IOP was artificially raised to a very high pressure (60–70 mm of Hg), the mean rise in IOP in eyes with glaucoma in our study when compared with healthy controls was small but was persistent. A reduction of 12.6% in ECC in the glaucoma patients might have been because of consistent raised IOP for a variable duration before they

visited our hospital when they were diagnosed as glaucoma. Moreover, in the current study, a decrease in ECC was significantly correlated with the severity of glaucoma at their first presentation [Table 4]. Severe glaucoma cases are expected to be having this disease lying undiagnosed for a longer interval of time. Moreover, the NTG group did not show a marked difference in endothelial parameters compared to healthy controls. We deduce that a persistent rise of IOP for a longer duration can affect the morphology of the corneal endothelium because of continuous mechanical insult. However, how long it takes for the small rise in IOP to begin to reduce the corneal endothelial cell density is not established.[12]

Endothelial cells are hexagonal and are arranged in a mosaic pattern such that they maintain a physical barrier and prevent aqueous substances from getting into the corneal stroma. They also have a pump mechanism that allows the stroma to remain anhydrous. Because of these functions, not only the cell count but also the morphology is essential in maintaining corneal clarity. In our study, there was a significant decrease in the percentage of hexagonal cells (48.40% in cases vs 68.68% in controls) and a significantly high CV (35.7) in glaucoma cases as compared to controls (27.06), indicating considerable pleomorphism and polymegathism in glaucomatous eyes. Bhomaj *et al*.[10] in their study also found that endothelial cells in normal eyes had a more uniform morphology evidenced by the lower CV of cell size and a higher proportion of hexagonality compared to eyes with glaucoma. Furthermore, we noticed that a change in the CV of cell size and percentage of hexagonality was more pronounced in cases of POAG and cPACG than in NTG patients when compared to controls. Sihota *et al*.[15] also found a statistically significant drop in the endothelial cell density in cPACG, with qualitative changes of pleomorphism and polymegathism.

Sung Woo Cho observed no significant difference in CV of cell size and hexagonality among NTG patients and the normal group.[12]

### Limitations

This study had some limitations. First, there is a possibility of sampling error because of the relatively small sample size. Second, we measured the IOP of patients during office hours, so a high IOP above 21 mmHg may have occurred in NTG patients outside of office hours. Third, we could not obtain information about the corneal endothelium of POAG patients before the first hospital visit. Therefore, it is not clear whether the reason for a low corneal endothelial cell density in eyes with POAG was the amount of elevated IOP or other factors such as congenital problems. We believe that a large population-based prospective study is needed.

## Conclusion

In conclusion, our study confirmed that there is a significant decrease in corneal endothelial cell density in eyes with POAG and cPACG. However, there is no significant decrease in corneal endothelial density in eyes with NTG. Therefore, elevated IOP is likely to cause a decrease in corneal endothelial cell density in eyes with glaucoma.

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### Conflicts of interest

There are no conflicts of interest.

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