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Assessment of Changes in Corneal Endothelial Characteristics in Primary Open-Angle Glaucoma

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ABSTRACT

Patients with glaucoma undergo significant changes in corneal endothelial characteristics due to chronically elevated Intraocular pressure (IOP). **Objectives:** To compare endothelial cell density between primary open-angle glaucoma (POAG) patients and age-matched non-glaucomatous controls. Also to explore the relationship between endothelial cell density and Intraocular pressure. **Methods:** This case-control study was conducted at Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan. It included 41 eyes of patients with POAG aged between 35-70 years and 41 eyes of age-matched non-glaucomatous subjects were taken as controls. The POAG was diagnosed based on Intraocular pressure, optic disc changes, and visual field defects. All participants went through a comprehensive ocular evaluation, that included slit-lamp examination, gonioscopy and Intraocular pressure assessment. The endothelial cell density was assessed via specular microscopy. SPSS version 26.0 was utilized to perform statistical analysis. **Results:** The average corneal endothelial cell density in healthy control subjects was 2484.51 ± 286.44 cells/mm^2, but those with POAG showed a statistically significant decline, measuring 2345 ± 270.29 cells/mm^2 (p=0.02). A notable decrease in endothelial cell density was seen in patients using dorzolamide 2262.00 ± 287.15 relative to patients not using dorzolamide 2451.28 ± 209.56 (0=0.02). Endothelial cell density and the average Intraocular pressure revealed a weak inverse correlation (r= -0.204, p=0.06). **Conclusions:** It was concluded that POAG patients show reduced corneal endothelial cell density. It also suggests that endothelial cell density declines with higher Intraocular pressure and increased disease severity, making it a possible biomarker of disease progression in POAG.

INTRODUCTION

Glaucoma consists of a range of conditions that are defined by characteristic patterns of damage to the optic disc and changes in the nerve fiber layer of the retina [1-3]. Research has shown that the changes in corneal endothelial cell density (ECD) are linked to glaucoma, with endothelial cell loss seen in different varieties of glaucoma such as POAG, primary angle-closure glaucoma [4], and certain types of secondary glaucoma [5]. Globally, POAG is one of the chief contributors to irreversible blindness worldwide [6, 7]. It is characterized by progressive damage of the optic nerve and characteristic loss of visual fields, frequently associated with elevated Intraocular pressure (IOP) [7]. Although there has been significant progress in understanding and managing POAG, the primary mechanisms driving its progress remain not fully understood. Whereas attention has been predominantly focused on the effect of IOP on the retinal nerve fiber layer and optic nerve, the effect on other ocular tissues, such as the corneal endothelium, has not been fully addressed. The cornea is an optically clear, avascular, and highly innervated structure. The innermost layer of the cornea is composed of single-layered polygonal endothelial cells that make up the endothelium. These cells are crucial for preserving corneal hydration and clarity by ensuring the movement of fluids and solutes between the aqueous humor and cornea [8]. At birth, the average corneal ECD is approximately 3,000 cells/mm2, and by adulthood, it decreases to approximately 2,500 cells/mm2 [9]. The regenerating ability of these endothelial cells is limited, and 400–500 cells/mm2 is the critical ECD needed to sustain

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the endothelial pumping function [10]. Reduced visual acuity, corneal edema formation, and decreased corneal transparency can all occur when ECD falls below the threshold value. Corneal endothelial cell (CEC) loss can result from trauma, intraocular surgery [11, 12], or conditions including diabetes [13]. CECs are particularly vulnerable to oxidative stress, inflammation, and elevated IOP, which are characteristic of glaucoma [9]. Assessment of the status of CECs is important in planning glaucoma treatment. Antiglaucoma medications particularly topical carbonic anhydrase inhibitors(CAIs) have been found to affect CECs by different mechanisms [14, 15]. Topical CAIs such as brinzolamide and dorzolamide, are commonly used for reducing IOP in the management of glaucoma. However, their effect on ECD and central corneal thickness (CCT) has been a debate of controversy [16, 17]. CAIs change the aqueous humor dynamics and pH levels, and this could impact the corneal endothelium. For patients undergoing anterior segment surgery, understanding ECD is important since eyes with lower cell densities are more likely to suffer from post-op corneal decompensation compared to normal eyes [11, 12].

Despite growing evidence that primary open-angle glaucoma (POAG) primarily damages the optic nerve, limited local research has explored its effect on corneal endothelial characteristics, particularly endothelial cell density (ECD), in Pakistani populations. Previous studies have reported inconsistent findings regarding the relationship between intraocular pressure (IOP), glaucoma severity, antiglaucoma medications such as dorzolamide, and corneal endothelial damage. This creates a significant clinical gap in understanding whether ECD can serve as an early biomarker for disease progression and treatment-related corneal compromise. This study aims to investigate the changes in the density of CECs in POAG. Earlier studies have implied a possible link between POAG and decreased ECD, though results have been inconsistent [4, 18]. This study also examines the relationship between IOP and various characteristics of CECs, considering the potential of using ECD as a biomarker for POAG progression.

METHODS

This case-control study was conducted at the Glaucoma Department of Al-Shifa Trust Eye Hospital (ASTEH), Rawalpindi, from June 2024 to December 2024.82 individuals were inducted in the study, of whom 41 were POAG cases and 41 were age-matched healthy controls. The age of participants ranged from 35 to 70 years, both male and female. Only one eye from each participant was considered for the study. The sampling method to select the participants was a non-probability consecutive sampling method.The research conformed to the regulations of the Helsinki Declaration and permission was given by the Ethical Review Committee at Al-Shifa Research Centre (approval number:ERC-16/AST-24).All participants received thorough counselling regarding the study and gave informed consent before enrollment. The sample size for this study was measured by power analysis utilizing Open Epi software version 3.01[19], using the mean and standard deviations provided in the reference study by Yu ZY *et al.*, with a mean of 2959 ± 236 cells/mm2 and 2757 ± 262 cells/mm2 between the two groups keeping the power of the study at 95% and confidence interval at 95% [18]. The analysis indicated that a minimum of 40 participants per group was necessary to reliably observe the mean difference of 202 cells. To account for potential attrition and data inconsistencies, an additional participant was included per group yielding a final sample size of 82 participants. Intraocular pressure (IOP) readings of greater than 21 mm Hg on a Goldman applanation tonometer on three or more occasions, glaucomatous optic disc changes (a cup-disc ratio (CDR) greater than 0.4 or an inter-eye CDR disparity greater than 0.2), and the identification of distinctive visual field defects using the Humphrey Visual Field (HVF) Analyzer (Carl Zeiss) were the criteria used to diagnose POAG in Group 1. Glaucoma severity was defined as early (MD> -6 dB), moderate (MD -6-12 dB), or advanced (MD> -12 dB) on the Humphrey visual field test. Each participant recruited in the POAG group showed visual field loss (mean deviation> -6dB) on at least two consecutive automated perimetric tests. Posner 4-mirror goniolens was used for gonioscopy, Shaffer grading system was used for anterior chamber angle assessment. Gonioscopy confirmed open anterior chamber angles (Shaffer grades 3 or 4). The control group included healthy individuals who had no glaucoma symptoms or indicators, had no pertinent eye history, and had no corneal pathology or any systemic illness that could affect CECs [13]. Their optic discs and visual fields showed no abnormalities, and the IOP readings on the Goldman application tonometer were all below 21 mmHg on more than two occasions. The exclusion criteria comprised individuals with a track record of corneal pathologies, use of contact lenses, ocular trauma, previous ocular operations (including intraocular surgeries or laser procedures), congenital ocular disorders, cataracts, spherical equivalent refractive error larger than ± 6 diopters, or systemic conditions as diabetes mellitus as these conditions could limit the generalizability of the study [13]. Moreover, any disease that could independently affect ECD was deemed a basis for exclusion [13]. Participants received a thorough ophthalmic evaluation, which included detailed ocular and systemic history, best corrected visual acuity (BCVA) using the Snellen acuity Chart, anterior and posterior segment slit-lamp examination, and dilated fundus biomicroscopy utilizing a +90 Diopter lens. Goldmann Applanation Tonometry (GAT) was used to assess the IOP.nPosner 4-mirror goniolens was used for gonioscopy to verify open-angle condition. Corneal endothelial cell (CEC) characteristics were evaluated with specular microscopy EM-4000, Tomey

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Corporation, Nagoya, Japan. Parameters such as ECD, Standard Deviation (SD) of mean cell area, Maximum Cell Area (MAX), Minimum cell area (MIN), average cell Area (AVG), Coefficient of Variation of cell size (CV), and percentage of cell hexagonality (6A) were recorded. IBM SPSS software for Windows, Version 26.0 2019; IBM Corp was utilized for statistical analysis.To summarize demographic and clinical data, Descriptive statistics were applied. The normality of the continuous data was evaluated before application of statistical tests. Independent t-tests were used to compare the group means for normally distributed data. Pearson's correlation coefficients were used to measure the correlations between clinical measures, such as IOP and ECD, IOP and MAX, IOP and MIN, IOP and AVG, IOP and 6A. Statistical significance was set at $p<0.05$ with 95% confidence intervals(CI).

RESULTS

The study comprised 41 patients diagnosed with POAG with a mean age of 54.49 ± 9.24 years including 51% male and 49% female, and 41 age-matched healthy controls with a mean age of 50.27 ± 7.70 years including 46% male and 54% female. The mean IOP in the POAG cohort was 17.98 ± 5.14 mmHg, while in the control group, it was 14.37 ± 2.52 mmHg, with a mean difference of 3.61 mmHg. The corneal ECD in the POAG group (2345.10 ± 270.29 cells/mm^2) was inferior to that of the control group (2484.51 ± 286.44 cells/mm^2) indicating that reduction in CECs was associated with raised IOP in POAG. Based on these patients' hospital records, the median length of glaucoma was 14.05 months (IQR:22.5 months). Key demographic and clinical characteristics showing notable differences in IOP, and ECD between the 2 groups (Table 1).

Table 1: Summary of Key Demographic and Clinical Differences Between the 2 Groups

Characteristics	Group A (POAG) n=41	Group B (Control) n=41	p-Value
Age (years)	54.49	50.27	0.03
Male: Female %	51:49	46:54	<0.01
Cup-Disc Ratio	0.6	0.2	<0.01
Mean IOP (mmHg)	17.98	14.37	<0.01
Average ECD (cells/mm^2)	2345.10	2484.51	0.03
Duration of Glaucoma (Months)	14 (IQR 22.5)	-	-

Specular microscopy gives important information about the general physical state of the CECs which includes size, shape and density.This is a non-invasive diagnostic technique. It helps to identify and manage various corneal conditions. In this study, the specular microscopy showed that the ECD was significantly reduced in POAG patients (2484 ± 286 cells/mm2) in comparison to the control group (2345 ± 270 cells/mm2)(p=0.026) with 95% CI(-261.81,-17.01) suggesting that the impact on ECD in POAG may be more directly related to the raised IOP. Patients with glaucoma had a considerably higher average cell area (AVG)(452.83 ± 49.71 µm2) than the control group (418.93 ± 57.56 µm2) (p<0.01) with 95% CI (10.26, 57.54). This refers to the variation in the area of the endothelial cells under stress, due to raised IOP, which results in the loss of normal hexagonal structure and increase in average cell area. Similarly, SD, MAX and MIN were higher in POAG patients. Patients with POAG had a lower percentage of hexagonal cells 6A (49.78 ± 3.25) than controls (51.37 ± 3.05) (p=0.03) with 95% CI (-2.97, -1.98). This finding suggests that patients with POAG lost the normal hexagonal structure of CEC possibly due to chronic insult by elevated IOP. The CV did not significantly change between the two groups (Table 2).

Table 2: Summarized CEC Characteristics of the POAG and the Control Group

Characteristics	Group A (POAG) n=41	Group B (Control) n=41	p-Value	Mean Difference	95%Confidence Interval, CI Lower Limit	95%Confidence Interval, CI Upper Limit	Effect Size (Cohen's *d*)
ECD, (cells/mm^2)	2345.10 ± 270	2484.51 ± 286	0.02*	-139.41	-261.81	-17.01	0.50
SD (µm^2)	164.12 ± 34.65	146.88 ± 24.38	0.01*	17.24	4.07	30.41	0.57
CV (%)	37.17 ± 6.82	36.07 ± 4.08	0.37	1.09	-1.37	3.57	0.19
6A (%)	49.78 ± 3.25	51.37 ± 3.05	0.03*	-1.58	-2.97	-1.98	0.50
AVE (µm^2)	452.83 ± 49.71	418.93 ± 57.56	<0.01*	33.90	10.26	57.54	0.63
MAX (µm^2)	1036.80 ± 272.06	926.10 ± 171.80	0.03*	110.70	10.70	210.70	0.48
MIN (µm^2)	114.32 ± 27.99	99.29 ± 30.90	0.02*	15.02	2.06	27.98	0.50

Note: *indicates a statistically significant difference at p<0.05

The 41 patients with POAG were further categorized into dorzolamide-untreated group (n=18) and dorzolamide-treated group (n=23). There was no major difference in age and gender between these two sub-groups. Stratifying POAG patients based on dorzolamide treatment revealed a mean ECD of 2262.00 ± 287.15 cells/mm^2 for treated patients and 2451.28 ± 209.56

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cells/mm^2 for untreated patients. Statistical analysis confirmed a significant reduction in ECD among those treated with dorzolamide (p=0.026, Cohen's d=0.75), while other parameters, such as percentage of hexagonal cells and average cell area, did not show statistical significance. A detailed comparison of endothelial cell parameters between patients treated with dorzolamide and those not treated with dorzolamide is provided (Table 3).

Table 3: Summary of CEC Characteristics among Dorzolamide-Treated and Untreated Patients

Characteristics	Dorzolamide -Treated, n=23	Dorzolamide-Untreated, n=18	p-Value	Mean Difference	95%Confidence Interval, CI Lower Limit	95%Confidence Interval, CI Upper Limit	Effect Size (Cohen's *d*)
ECD (cells/mm^2)	2262.00 ± 287.15	2451.28 ± 209.56	0.02*	-189.27	26.17	352.38	0.75
6A (%)	49.78 ± 3.45	49.78 ± 3.07	0.99	0.05	-2.10	2.09	0.0
AVE (µm^2)	450.96 ± 48.42	455.22 ± 52.63	0.78	-4.26	-27.75	36.28	0.08
MAX (µm^2)	1055.22 ± 283.24	1013.28 ± 263.24	0.63	41.94	-216.79	132.91	0.15
MIN (µm^2)	118.65 ± 32.12	108.78 ± 21.21	0.26	9.87	-27.63	7.88	0.36

Note: *indicates a significant difference at p<0.05

A weak opposite correlation was observed between IOP and ECD (r= -0.204, p=0.06). However, only the positive trend between the IOP and maximum cell area was found to be statistically significant (p=0.001)(Figure 1).

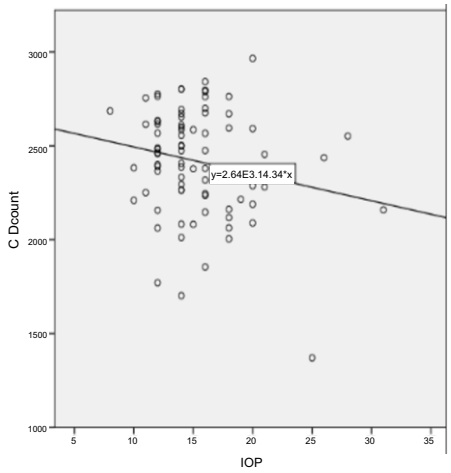

Figure 1: Correlation Between IOP and ECD

Positive relationships were identified between IOP and Average Cell Area (r=0.202, p=0.06). However, only the positive trend between the IOP and maximum cell area was found to be statistically significant (p=0.001)(Figure 2).

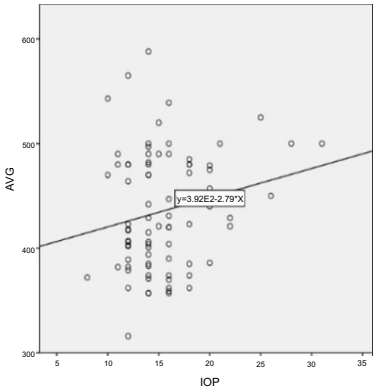

Figure 2: Correlation Between IOP and Average Cell Area

Positive relationships were identified in IOP and Maximum Cell Area (r=0.345, p=0.001)(Figure 3).

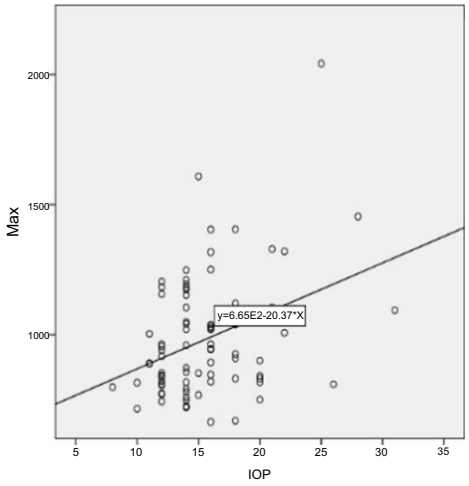

Figure 3: Positive Relationships Between IOP and Maximum Cell Area

Positive relationships were identified in IOP and Minimum Cell Area (r=0.182, p=0.10)(Figure 4).

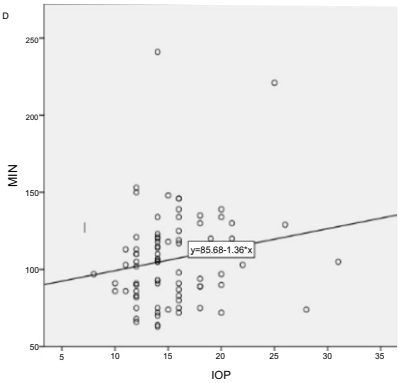

Figure 4: Positive Relationships Between IOP and Minimum Cell Area

No substantial association was identified between IOP and the percentage of hexagonality (r=-0.01, p=0.92)(Figure 5).

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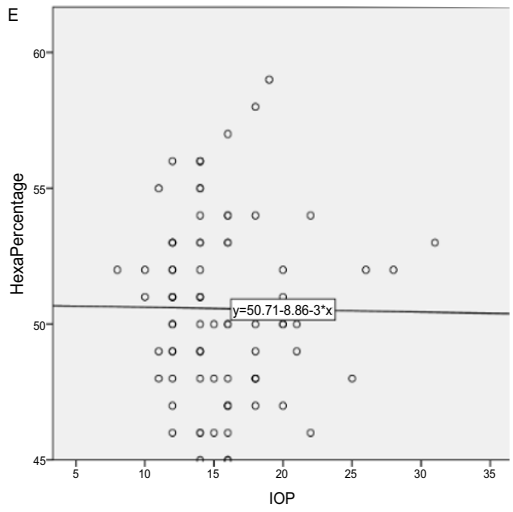

Figure 5: Association Was Identified Between IOP and the Percentage of Hexagonality

DISCUSSION

ECD is a major indicator of endothelial health. The lower count indicates a damaged cornea. Polymegathism is the variation in cell size measured by coefficient of variation, CV. pleomorphism is the percentage of hexagonal cells. Both factors provide valuable insights about the regularity and survival of endothelial cells. The higher coefficient of variation indicates higher variation and frequently indicating cellular stress or disease [20]. The lower percentage of hexagonal cells shows increased cell shape irregularity which could point to compromised endothelial layer. Chronically elevated IOP in POAG is associated with changes in corneal endothelial characteristics, including reduced ECD, increased pleomorphism, and polymegathism. The objective of this study was to find out if there was any variation in the corneal endothelial characteristics in subjects diagnosed with POAG compared with healthy participants. The corneal endothelium is a single layer of cells with hexagonal structure that ensure clarity of cornea by controlling fluid and solute exchange. The susceptibility of this layer to IOP-related stress underscores its importance in overall ocular integrity [21]. These cells possess limited regenerative capacity, thus being severely susceptible to injury from prolonged mechanical stress brought on by high IOP [21]. High IOP directly applies mechanical stress on the corneal endothelium. This stress can lead to cell deformation, rupture of cell junctions, and loss of its function in maintaining corneal deturgescence. Chronically high IOP causes cellular apoptosis in the endothelium due to oxidative stress and inflammation. This results in progressive loss of ECD with time [22]. The prolonged exposure to high IOP could interfere with the metabolic activity of endothelial cells, impairing their function to keep the cornea optically clear[8]. In our research the decrease in ECD seen in POAG patients is found to be consistent with the previous studies that endothelial cell damage is influenced by persistently high IOP, a characteristic of POAG [18, 4]. The term polymegathism, or coefficient of variation, refers to the variation in the cell areas of individual cells. 100% of the cells in a healthy cornea should be hexagonal in shape. It is anticipated that a typical cornea will have hexagonality of 60%. Under stress or insult to the endothelium, as seen in raised IOP, hexagonality decreases and cell area increases [20]. According to our study patients with POAG had a statistically significant reduction in % of hexagonal cells (p=0.03) and increase in average cell area(p=<0.01) indicating stress induced loss of endothelial cells with resultant increase in the size of remaining viable cells and loss of normal architecture. A positive correlation was identified between the IOP and maximum cell area (p=0.001), suggesting an overall enlargement of endothelial cells which is frequently associated with reduction in ECD due to damage to the corneal endothelium. The endothelial damage is thought to be repaired by elongation and spread of the remaining cells, to generate a consistent cellular layer across the inner surface of the cornea [20, 23]. This causes the number of endothelial cells to decrease and the surface area to grow as evident by the findings in our study. This study suggests that the impact of POAG on the corneal endothelium may be more directly related to disease-specific factors such as IOP and the use of certain IOP-lowering agents which further can lead to endothelial cell damage. Reducing intraocular pressure is the only treatment that has been proven to be effective and is widely recognized for halting the progression of glaucoma [24]. The mainstay of POAG treatment to reduce intraocular pressure is with topical medications, laser therapy, and glaucoma surgery. However, medical treatment with topical eye drops is regarded as an appropriate first line of therapy in published guidelines for the treatment of POAG [25].Different anti-glaucoma medications are available that can be applied topically to reduce IOP. Their modes of action and the extent to which they reduce intraocular pressure vary [26].A network meta-analysis of topical first-line medications found that prostaglandin analogues reduce intraocular pressure the most, followed by beta-blockers, alpha2-adrenergic agonists, and carbonic anhydrase inhibitors(CAIs)[26]. The possible impact of medications on corneal endothelium is a matter of concern even with proven advantages of glaucoma pharmacotherapy in lowering IOP [26].The findings of the investigation to find a correlation between different types of anti-glaucoma medication and corneal endothelial damage have not been consistent [14,15]. Dorzolamide was the first commercial product approved by the Food and Drug Administration (FDA) for the treatment

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of glaucoma [27]. It is widely used in the management of POAG. Many clinical studies agreed that the use of topical dorzolamide had no meaningful effect on endothelial cell count and cell shape [14, 15]. However, Lass *et al.*, contradict this finding, showing that after one year of topical dorzolamide therapy in individuals with POAG or ocular hypertension, the mean percent CEC loss was 3.6% [28]. In our study topical instillation of dorzolamide in POAG patients has been shown to cause a statistically significant reduction in ECD. However, it has not been shown to cause any statistically significant effect on corneal endothelial cell size or shape. A reduction in ECD may indicate poor IOP control and the need for adjustments in therapeutic strategies. This is particularly significant because patients with POAG who do not respond well to topical medication need surgical intervention to halt glaucoma progression. These patients face an increased risk of further CEC loss, leading to reduced corneal clarity, as evidenced by multiple studies [11, 12]. Because an unhealthy endothelial layer can have serious postoperative consequences, for ophthalmic procedures including cataract extraction and trabeculectomy [29], the major determining factor in selecting patients and surgical prognosis is endothelial integrity. This study emphasizes the importance of corneal endothelial assessment in POAG patients undergoing intraocular surgical procedures. Nevertheless, the role of ECD as a biomarker for glaucoma progression should be tested thoroughly with extensive research. A large sample size would generalize the findings of the current study. A more diverse demographic approach could provide insights into how various factors like ethnicity or lifestyle influence ECD in glaucoma patients. A longitudinal study design would help in understanding the progression of ECD changes over time. Further studies are needed to track the long-term impact of elevated IOP on endothelial health and the effect of different glaucoma treatments on ECD for safer therapeutic practices. Systemic conditions that are known to contribute to the reduction of endothelial count, like diabetes mellitus [20-22], were not the focus of this study and require further investigation. This research study focuses on the necessity of integrating ECD monitoring into glaucoma treatment protocols and emphasizes the cautionary use of dorzolamide. Ongoing research into pharmacotherapies with less endothelial toxicity and a protective effect on endothelial cells could improve outcomes for glaucoma patients.

The study was limited by its single-center design, relatively small sample size, and cross-sectional nature, which restricted broader generalizability and prevented long-term assessment of endothelial changes over disease progression. Additionally, the influence of systemic comorbidities and duration-specific medication effects may not have been fully captured. Future multicenter longitudinal studies with larger, more diverse populations are recommended to validate ECD as a reliable biomarker for POAG progression and to further investigate the long-term safety of various glaucoma therapies on corneal endothelial health.

CONCLUSIONS

It was concluded that this study shows a decline in the density of CECs in patients with POAG, demonstrating that a lower ECD is associated with more severe disease and higher IOP. Our findings highlight the need for corneal endothelial monitoring in glaucoma management, particularly in advanced cases or those with poorly controlled IOP. The reverse correlation between ECD and IOP emphasizes the need for stable IOP control to protect corneal endothelial function. Future longitudinal studies should investigate ECD reduction mechanisms in POAG and assess its potential as a biomarker for disease progression.

Authors' Contribution

Conceptualization: TT
Methodology: TT, AA[1], NR, AA[2], KH
Formal analysis: TT, FA
Writing and Drafting: MA
Review and Editing: MA, TT, FA, AA[1], NR, AA[2], KH

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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