ORIGINAL ARTICLE

Case-Control Study of Corneal Endothelial Cell Density in Primary Open-Angle Glaucoma Patients Versus Normal Population Using Specular Microscopy in Rawalpindi, Pakistan

Sania Sajjad[1*], Abdul Rauf[2], Muhammad Kamran Haider[2], Syeda Muniba Zahra[2], Muhammad Saad Naseer[2], Farhat Zahra[2]

ABSTRACT

Objective: To assess Endothelial cell counts in the corneas of patients with primary open-angle glaucoma (POAG) as compared to age-matched healthy individuals using Corneal Specular Microscopy.

Study Design: Case-control study.

Place and Duration of Study: This study was conducted at the Armed Forces Institute of Ophthalmology (AFIO), Rawalpindi, Pakistan from December 2023 to May 2024.

Methods: A total of 50 individuals were recruited, including 25 phakic Primary Open Angle Glaucoma patients on antiglaucoma medications for at least five consecutive years. They were compared with 25 healthy phakic individuals as controls. All the individuals were aged 50-60 years. Intraocular pressure (IOP) was recorded using Goldmann Applanation Tonometry (GAT). Endothelial cell density was measured using Indirect Specular Microscopy SP-3000P® in both groups after thorough ophthalmological examination. SPSS 25 was used for statistical analysis. Age was stratified into 3-year intervals from 50 to 60 years. Paired sample t-test was employed to assess the mean difference, and a *P*-value <0.05 was considered statistically significant.

Results: Among included participants (N=50), 26 (52%) were males and 24 (48%) were females. Participants had a mean age of 55.08 ± 2.48 years. The mean endothelial cell count in Primary Open Angle Glaucoma patients was 2067±155 cells/mm² compared to controls 2715±315 cells/mm² (*P*=0.0001). There was no statistically significant difference in endothelial cell counts (in cells/mm²) between males and females or across stratified age groups. (*P* > 0.05)

Conclusions: Primary Open Angle Glaucoma patients exhibit significantly reduced endothelial cell density compared to healthy individuals. Chronic intraocular pressure elevation and long-term exposure to anti-glaucoma medications may contribute to endothelial loss of cells.

Keywords: *Corneal Endothelium, Endothelial Cell Count, Glaucoma, Open-Angle, Specular Microscopy, Tonometry.*

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Introduction

The cornea has six layers, of which the endothelium is considered the most important. It is a single layer of cells situated beneath Descemet's membrane and plays a pivotal role in maintaining corneal clarity by pumping fluid out of the stroma into the anterior chamber. It is approximately 5 microns in thicknessand hexagonal cells are arranged in, with hexagonal cells arranged in a honeycomb pattern.[1] The number of endothelial cells in adults averages between 1500 and 2500 per mm² and decreases with age at a rate of 0.3–0.6% per year.[2] When this number falls below 600, the cornea accumulates

[1]Department of Ophthalmology
Combined Military Hospitals (CMH), Multan, Pakistan
[2]Department of Ophthalmology
Armed Forces Institute of Opthamology (AFIO), Rawalpindi, Pakistan

Correspondence:
Dr. Sania Sajjad
Department of Ophthalmology
Combined Military Hospitals (CMH), Multan, Pakistan
E-mail: saniaabbasi555@hotmail.com

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aqueous humour inside it and loses transparency.[3] A non-contact, non-invasive technique known as Specular microscopy is the new modality used to visualize and estimate endothelial cell count.[4] It is routinely done and crucial in mapping and preoperative endothelial health assessment, especially before cataract surgery, and donor cornea before corneal transplantation.[5] Similarly, there are various corneal pathologies where specular microscopy plays an important role in diagnosis and prognosis in pseudophakic bullous keratopathy, endothelial dystrophy of Fuchs', CHED(congenital hereditary endothelial dystrophy), viral endothelitis, and trauma.[6]

Glaucoma is an umbrella term that is characterized by optic neuropathy and corresponding progressive visual field defects, with IOP as a variable modifiable risk factor.[7] Increasingly, we find evidence of glaucoma patients (angle-closure , primary open-angle(POAG), and secondary glaucoma patients) presenting with corneal endothelial cell loss.[8] Various theories are suggested that explain the decrease in endothelial cell counts of the cornea in POAG due to the direct compression effect in angle closure, with the toxicity of anti-glaucoma medication (especially topical carbonic anhydrase inhibitors, preservatives, and the use of antiproliferative medicines like mitomycin and 5-FU in glaucoma filtration surgeries) and the implantation of glaucoma drainage devices.[9,10] Evaluation of endothelial count in these patients is extremely important before cataract extraction. Cataract surgery is already challenging in a glaucomatous eye due to various factors, including poor pupillary dilation, shallow AC depth, fragile lens capsule, and zonules.

In this study, we aimed to compare the endothelial cell density in glaucoma patients and its correlation with intraocular pressure control with a normal population of a similar age group using specular microscopy.

Methods

This study took place over six months from December 2023 to May 2024 as a comparative case-control study. A total sample size of 16 with a case-to-control ratio of 1:1 (8 in each group) was calculated using OpenEpi Software online for Case-control studies, keeping a confidence interval of 95%, a power of 80% and a mean difference of 380.[11] However, we included all patients fulfilling the inclusion-exclusion criteria during the study period, N=50 (i.e., 25 cases and 25 controls). Permission of the hospital Ethical Review Committee was granted vide letter no: 317/ERC/AFIO, held on dated: 23rd June 2023. Consent was obtained from all included participants before the study.

Inclusion Criteria: We enrolled individuals of POAG of both genders and age group between 50-60 years with at least 5 years history of Primary open-angle glaucoma and using Anti-Glaucoma drugs only: one group and healthy phakic individuals in the other groups.

Exclusion Criteria: Participants were excluded if they had primary or secondary angle closure glaucoma or secondary open-angle glaucoma, had undergone laser trabeculoplasty, peripheral iridotomy, glaucoma filtration surgeries, previous ocular trauma, previous ocular surgery or corneal pathologies which affect endothelium like Fuchs corneal dystrophy, corneal dystrophies, pseudophakic bullous keratopathy or if there was history of any ophthalmic disease.

A non-probability consecutive sampling technique was employed. 25 participants who were already established glaucoma patients, having RNFL damage on OCT with corresponding visual field loss and grade 3 or 4 angle on von Herick grading, and already using antiglaucoma drugs for 5 years, were selected from the Glaucoma filter clinic. Similarly, 25 participants were enrolled from the General OPD who met the inclusion criteria. Each participant underwent a comprehensive ophthalmic evaluation, including intraocular pressure measurement and fundus examination with a Volk Superfield lens. The IOP of all subjects was measured by the same examiner using Goldmann applanation tonometry. The eye examined was anaesthetized using a topical Anasthetic (Alcaine eye drops) and fluorescein strips were used to stain the cornea and tear film. 3 readings were taken on GAT with Slit Lamp between 1200 hrs and 1400hrs to minimize any potential variations due to diurnal variations and the average value was determined and used for analysis.

After dividing into groups, the POAG and Control groups, Indirect specular microscopy SP-3000P (TOPCON, Tokyo, Japan) was performed on each subject. Images were captured from the central cornea and analyzed using ImageNet 3000 software, which automatically detected endothelial cell counts. Endothelial cell density was determined from the printout of the document. All examinations were performed by the same resident ophthalmologist to maintain consistency.

After the collection of data, data was analyzed using the Statistical Package for Social Sciences (SPSS) version 25. Data was recorded by another resident ophthalmologist who was blinded to this study to minimize bias. The normality of data was assessed, and data were found to be normally distributed. The qualitative variable (gender) was expressed as frequency and percentage, while the quantitative variables (Age, endothelial count) were expressed as mean and standard deviation. For confounding, age was converted into 3-year interval ranges and expressed as groups. The Independent sample t-test was used to assess the statistical significance, and a *P* value of <0.05 was considered statistically significant.

Results

Fifty participants meeting the inclusion and exclusion criteria were included in the study. Twenty-five participants had POAG, and 25 participants were age-matched controls. The mean age was 55.08 ± 2.48 years, with 26 (52%) participants being males and 24 (48%) being females. The mean endothelial cell count was 2418 ± 359. The mean endothelial cell count in the POAG (group A) was 2067± 155, compared to the Control group (group B) 2715 ± 315. (Figure 1).

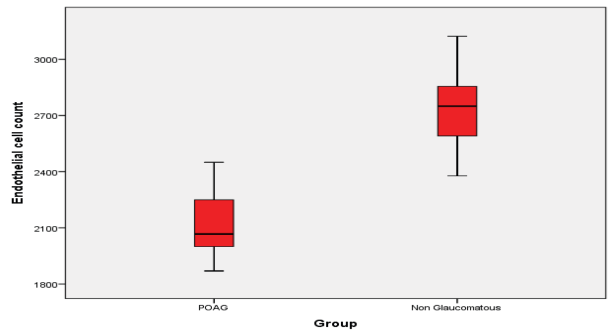

Fig.1: Stem and Leaf plot showing the endothelial cell counts in the POAG vs the Control group. Note the values are significantly lower in primary open-angle glaucoma (POAG)

Endothelial cell count was found to be significantly lower in the POAG group as compared to the control group (*P* =0.0001). There was very strong and statistically significant correlation (r = 0.854, *P* = 0.000) between POAG status and reduced endothelial cell counts. Mean endothelial cell count in males was 2386 ± 351, while females had 2454 ± 371. There was a significant difference between endothelial cell counts among genders and age groups (*P*< 0.001). There was no significant correlation of age, Gender, and Age Groups with Endothelial cell counts. (Table 1.)

Table 1: Mean Endothelial Cell Counts summary statistics

Endothelial Cell Counts (N=50)		Endothelial cell count Mean	SD	Correlation (r)	*t*-test value	*P* value
Group	POAG (N=25)	2115	155	0.854	-11.37	0.001
	Controls (N=25)	2723	217			
Gender	Male (N=26)	2386	352	0.095	-0.54	0.590
	Female (N=24)	2454	372			
Age Groups	50-53 years (N=14)	2286	280	0.245	-1.279	-1.279
	54-56 years (N=20)	2436	370			
	57-60 years (N=16)	2513	393			

Discussion

Endothelial cells serve the vital function of preserving corneal integrity and clarity. Our study highlights that there was a significant reduction in endothelial cell density in patients with POAG when compared to matched controls. The endothelial cell counts were significantly lower (2067 ± 155 cells/mm²) in the POAG group compared to the control group (2715 ± 315 cells/mm²). This reduction aligns with previous studies demonstrating that glaucoma patients exhibit a marked decline in endothelial cell counts. Vaiciuliene R et al. suggest chronic IOP elevation contributes to progressive endothelial dysfunction. They attribute this to increased levels of inflammatory proteins, particularly C4 and complement factors.[1] Realini T et al. reported a similar decrease in endothelial cell and primarily attributed it to prolonged exposure to anti-glaucoma medications.[8] They also express a similar decline in other glaucoma therapies. However, the mechanism of endothelial cell loss in POAG patients is multifactorial. One of the important factors is elevated IOP. Increased IOP leads to mechanical stress on the corneal endothelium from inside, which may potentially lead to apoptosis and functional impairment.[12] Additionally, the use of topical anti-glaucoma medications, particularly Carbonic Anhydrase Inhibitors (CAH) and Prostaglandin Analogues (PG), has been implicated in endothelial toxicity, which might be attributable to preservatives included in these drugs. However, studies show no effect of drugs on corneal endothelia.[13,14] However, glaucoma medication usage is an important confounding factor, considering many patients have different IOP levels and variable compliance. Similarly, the control group's usage of over-the-counter eye drops cannot be controlled in our study.[14]

Thatte S et al. have also reported similar reductions in endothelial cell counts among glaucoma patients (1610 ± 225 cells/mm2) compared with the control group (2210 ± 236 cells/ mm2).[15] This is further corroborated by Sugumaran A et al. who also had significantly fewer endothelial cell counts in patients with glaucoma.[16] Similar to our study, both studies find no significant correlation between gender and endothelial cell count. This is consistent with studies suggesting that while ageing contributes to endothelial cell attrition, its effect is less pronounced than that of chronic glaucoma and its treatments.[17] However, as previously described, they may be attributable to the prolonged usage of medications or other surgical interventions.[18] This surgical intervention is not just confined to glaucoma surgery but all kinds of intraocular surgery cause decreased cell count.

Many other publications further corroborated this.[18] Therefore, routine endothelial assessment is a must in glaucoma patients. particularly before cataract surgery. Cataract surgery significantly decreases endothelial cell counts. Specular microscopy serves as a vital tool in preoperative evaluation, allowing ophthalmologists to anticipate potential postoperative complications such as corneal decompensation, especially in glaucoma patients. Given the already compromised endothelial reserve in glaucoma patients, surgical strategies should be tailored to minimize endothelial trauma.[19,20]

While we tried an extensive search of literature to find any studies where there was no significant difference in endothelial counts between glaucoma patients, evidence seems to be lacking. It is noteworthy to mention that a few studies did find that there was no significant difference in patients with normal tension glaucoma.[21] This could suggest that there are other factors beyond just IOP that cause endothelial cell loss. One such factor is treatment for antiglaucoma. However, not all studies involving Normal tension glaucoma had the same results, where studies showed normal tension glaucoma in fact had more endothelial cell loss than open-angle glaucoma.[22]

Automated specular microscopy counts tend to be overestimated. Therefore, confocal microscopy can be an additional tool in this aspect to confirm the endothelial cell counts, and there should be RCTs to measure endothelial cell counts with different machines.[23] Finally, many studies assessed the differences between Angle-closure glaucoma and POAG for cell counts, and some also assessed it in Possner-Scholsman syndrome.[23] However, all studies have established that endothelial cell counts decrease in all conditions with higher IOP.[24] IOP appears to be the main factor behind decreased cell counts but the role of other confounders cannot be ignored. Surgical interventions in minimally invasive glaucoma surgery, cataract, or any glaucoma surgery further predispose to endothelial cell count.[25]

Our study reaffirms the significant impact of POAG and its treatment on corneal endothelial cell density. There were many confounding variables, e.g. age was a confounding variable. Although we stratified the age into 3-year study it might not be a true representation, and further studies need to confirm the role of age, especially for older age individuals. Gender was the other confounding variable, which similarly had no difference. Finally, we did not quantify glaucoma medications and their roles in decreased endothelial cell counts. Finally, we used a single machine to check cell counts, so the effect of the machines cannot be ruled out. These aspects should be investigated further in studies that quantify these variables. Our study has several limitations. The sample size was relatively small. It limits the generalizability of the findings. Additionally, potential confounding factors such as systemic diseases, lifestyle habits, and genetic predispositions were not extensively analyzed. Future research with larger and more diverse sample sizes and Randomized controlled trials is needed to validate these findings.

Conclusion

Endothelial Cell Counts are lower in Primary open-angle glaucoma patients as compared to normal healthy individuals. These findings extended to both genders and did not significantly differ as compared to age. It was likely attributed to a prolonged increase in IOP in POAG patients in addition to the use of antiglaucoma medications.

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Author Contributions

SS: Writing the original draft, proofreading, and approval for final submission
AR: Conception and design of the work
MKH: Manuscript writing for methodology design and investigation
SMZ: Revising, editing, and supervising for intellectual content
MSN: Data acquisition, curation, and statistical analysis
FZ: Validation of data, interpretation, and write-up of results