

Comparison of Corneal Endothelium in Patients with Uveitis and Healthy Subjects Using Specular Microscopy

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ABSTRACT

Objective: To evaluate changes in corneal endothelium in patients with anterior uveitis by using specular microscopy and compare it with the corneal endothelium of healthy subjects.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan, from Jun 2018 to Jun 2019.

Methodology: This study included 65 eyes of 65 patients with anterior uveitis and 65 eyes of healthy subjects. Corneal endothelium was assessed in all participants of both Groups by using Specular microscopy. Corneal endothelial markers like central corneal thickness (CCT), cell density (CD), cell minimum area, cell maximum area, the average of cell size, percent of hexagonality (HEX%), coefficient of variation (CV) were compared in both Groups. The Uveitis Group was further subdivided into active and inactive Uveitis Subgroups, and corneal endothelium markers were also compared in these Subgroups.

Results: The mean endothelial cell density was 2626.0 ± 413.0 cells/mm² in patients with uveitis, whereas cell density was 2766.0 ± 327.0 cells/mm² in healthy subjects. The difference in endothelial cell density was statistically significant between the Groups ($p=0.03$). The difference between central corneal thickness, maximum cell area, average cell size and hexagonality values between Groups was also statistically significant, whereas there was no difference in terms of cell minimum area between these Groups. There was no statistically significant difference between inactive and active uveitis when corneal endothelial markers were compared between them ($p>0.05$).

Conclusion: There was an effect of uveitis on central corneal thickness, endothelial cell density, cell size, and morphology compared to healthy subjects, whereas there was no difference in corneal endothelial markers in patients with active uveitis and inactive uveitis.

Keywords: Anterior uveitis, Corneal endothelium, Specular microscopy.

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INTRODUCTION

The corneal endothelium is essential in keeping the cornea dehydrated and maintaining transparency. Endothelial cells in the cornea are hexagonal and have limited ability to regenerate.¹ There can be changed in endothelium due to intrinsic factors like ageing and genetics and various extrinsic factors like surgery and trauma.^{1,2}

The Standardization of Uveitis Nomenclature Working Group (SUN) defined anterior uveitis as inflammation of the anterior chamber.^{3,4} Anterior uveitis accounts for 50-90% of all uveitis cases. It can be associated with systemic disorders like ankylosing spondylitis, sarcoidosis, interstitial nephritis, vasculitis, and inflammatory bowel diseases.³ The role of HLA B27 in the pathogenesis of anterior uveitis has also been reported by many studies.⁵ Anterior uveitis also affects corneal endothelium and can cause band

keratopathy, iridocorneal adhesions, and keratic precipitates.⁶ This corneal damage is caused by cytokines released during anterior segment inflammation is very crucial,^{1,7} as it can lead to many complications when such patient undergo surgery later in life.⁷

Corneal endothelial involvement in uveitis has not been studied considerably, although it might help in finding the target of inflammation.^{8,9} There are certain markers which can depict corneal health like endothelial cell density, cell shape and size.¹ It is seen that corneal decompensation occurs when endothelial density falls to 400-600 cells/mm² and not only reduction in number but poorly functioning cells due to cellular pleomorphism is also very important.² These corneal endothelial markers can be evaluated by using specular microscopy which gives magnified view of corneal endothelium. It is a major diagnostic test in clinical practice.^{1,7} It not only assesses the health of the endothelium in corneal diseases but is one of the routine tests after corneal transplantation. In addition to this, corneal safety is also measured after surgical procedures by using specular microscopy.¹⁰

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Healthy Subjects Using Specular Microscopy

In specular microscopy, a corneal endothelium image is taken when light is refracted at the anterior corneal surface.⁹ It is a non-contact test that patients tolerate better than the previous contact method. Physicians prefer it as it is quicker and simple to use, requires no anaesthesia and has little risk of trauma or infection.¹ It requires no gel in vivo confocal microscopy to prevent light scattering at corneal epithelium. It can evaluate Keratic precipitates morphology as well.^{9,10} Current specular microscopes have software for auto-focus and built-in image analysis too.

This study highlighted the importance of evaluating corneal health in patients with uveitis and the ability of the endothelium to withstand any surgical intervention that is particularly required in such patients. Therefore, the objective of the study was to evaluate changes in endothelium in patients with uveitis by using specular microscopy and comparing it with healthy subjects' corneal endothelium.

METHODOLOGY

This comparative cross-sectional study was performed at the Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan, from June 2018 to June 2019. This study was carried out after Institutional Ethical Review Board permission (Certificate Number 202/ERC/AFIO). All steps were carried out according to the ethical standards of the Institutional Committee and the Declaration of Helsinki. Informed consent was taken from all participants in the study. The study included two Groups.

Inclusion Criteria: In the first Group, patients with active uveitis who were visiting the department for treatment and those who had inactive uveitis for up to three months after treatment and were being followed up were included. In the second Group, healthy individuals were randomly selected from visitors, outpatients and staff.

Exclusion Criteria: Patients with a history of ocular surgery, trauma, glaucoma, corneal dystrophy or keratoconus were excluded from the study.

It included 65 eyes of 65 patients with uveitis and 65 eyes of healthy subjects. The sample size was calculated by the WHO sample size calculator by keeping a 95% Confidence level of 5% error; the mean CD for Group-1 was 2540.0 ± 619.0 , and Group-2 was 2834.0 ± 413.0 . The total sample size came as 102; 51 for each Group.¹¹ In the uveitis Group, 42 patients had idiopathic uveitis, seven patients had Tuberculosis, five patients were diagnosed with Behcet's disease,

two patients had Fuch's uveitis, three patients had positive TORCH serology, four patients had rheumatoid arthritis and two patients were known to have psoriasis. Twenty-three patients had active uveitis, and 42 patients had inactive uveitis. In the Uveitis-Group, 16 patients had anterior and posterior segment involvement, whereas in 49 patients, only the anterior segment was involved, and there was no posterior segment activity.

Patient particulars like gender, age, symptoms, drug history and systemic diseases were recorded. A visual assessment was done. Detailed anterior and posterior segment examinations were performed using slit lamp examination, and all finding activity in the anterior and posterior chamber was recorded. Patients with active diseases were given treatment after examination; topical steroids to control inflammation and Cyclopentolate 1% to control pain were advised. All patients were advised regular follow-up in OPD. Similarly, healthy subjects were also examined, and visual acuity and the anterior and posterior segment was carried out to rule out any ocular disease.

Specular microscopy (Topcon SP-2000P Tokyo, Japan) was used to take corneal endothelium images of patients included in the study. Patients have explained the procedure and are positioned in comfortable postures. Then, they were instructed to fixate on the light in the microscope. A single image of the corneal endothelium was taken from the centre of the cornea of each eye by a specular microscope.

Specular microscopy was used to measure Endothelial cell density (CD), hexagonality (HEX), coefficient of variation (CV), minimum cell area (Min) and cell maximum area (Max), the average cell size (AVG) and central corneal thickness (CCT), values independently in the centre of the cornea by the same examiner. Similarly, healthy subjects explained the procedure and corneal endothelium images were taken from the centre of the cornea. Again, all endothelial markers were independently measured, including endothelial SD, CV, HEX, Max, Min, AVG and CCT.

Statistical Package for Social Sciences (SPSS) version 20.0 was used for comparing corneal endothelium in patients with uveitis and healthy subjects. Comparison of the variables between Groups was performed by using an independent sample t-test. Evaluation of relations between measured variables was performed by Pearson's correlation. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

It was seen that there was a statistically significant difference between the uveitis and healthy subjects Group in terms of CCT, CD, Max, and HEX values (Table-I). The mean age of patients in the uveitis Group was 39.98±15.40 years; while in healthy subjects, the mean age was 30.97±8.60 years ($p=0.001$). Both Groups had 44 males (67.7%) and 21 females (32.3%).

reported decreased endothelial cell count in patients with Fuch's uveitis in his study.¹² Reijo *et al.* suggested decreased corneal endothelium cell density in patients with herpes keratouveitis, and they attributed high IOP to be partly responsible for this decrease in cell density.¹³ Ornek *et al.* have reported other causes that can lead to decreased corneal endothelial cell density like pseudoexfoliation (2510.6±220) when compared to

Table-I: Specular Microscopy Results between Uveitis and Healthy Subjects (n=110)

Parameters	Uveitis Group (n=65) (Mean±SD)	Healthy Subjects (n=65) (Mean±SD)	p-value
Central corneal thickness (CCT)µm	516.0±35.0	502.0±33.0	0.020
Cell maximum area (Max) µm ²	836.0±17.0	699.0±23.0	0.001
Cell minimum area (Min) µm ²	119.0±47.0	119.0±31.0	0.990
Average cell size (AVG) µm	392.0±77.0	367.0±43.0	0.02
Cell density (CD) cells/mm ²	2626.0±413.0	2766.0±327.0	0.034
Hexagonality (HEX)%	54.0±10.0	57.0±9.8	0.086
Coefficient of variation (CV)	35.0±4.7	34.0±6.8	0.33

The difference between patients with active uveitis and inactive uveitis was measured, and it was observed that there was no statistically significant difference between the two Subgroups in terms of CCT, CD, Max, Min, AVG, and HEX, but there was a statistically significant difference in terms of CV between two Subgroups (Table-II).

control Group (2740.4±330) and some eyes after treatment with SLT (selective laser trabeculoplasty) 2488.2±348.¹⁴

We have observed increased AVG in patients with uveitis than in healthy subjects. Guclu *et al.* had similar observations regarding AVG in their study.¹

Stressful conditions in the eye cause changes in

Table-II: Specular Microscopy Outcomes between Active and Inactive Uveitis (n=110)

Parameters	Active Uveitis (n=65) (Mean±SD)	Inactive Uveitis (n=65) (Mean±SD)	p-value
Central corneal thickness (CCT) µm	517.0±33.0	516.0±33.0	0.836
Cell maximum area (Max) µm ²	836.0±164.0	836.0±178.0	1.00
Cell minimum area (Min) µm ²	110.0±40.0	124.0±51.0	0.08
Average cell size (AVG) µm	376.0±50.0	401.0±88.0	0.048
Cell density (CD) cells/mm ²	2690.0±312.0	2591.0±459.0	0.1528
Hexagonality (HEX) %	51.0±9.0	55.0±10.0	0.018
Coefficient of variation (CV)	37.0±4.8	34.0±4.3	0.003

In correlation analysis, there was significant negative correlation in age and CD in patients with uveitis ($r=-0.46$, $p=0.01$). However, no correlation was found in another Group between age and CD. There was a negative correlation between age and hexagonality in the healthy subjects Group ($r=-0.29$, $p=0.02$), whereas it was not significant in the other Group.

DISCUSSION

In our study, it is seen that there was decreased CD in patients with uveitis when compared to healthy subjects. Various studies have reported the changes in corneal endothelium resulting from uveitis. Alfawaz *et al.* had reported that there was a lower CD in patients with uveitis when compared to normal individuals.⁶ Similarly, CT Pillai has described changes in endothelium in patients with active uveitis in the vicinity of keratic precipitates.⁷ Ozer *et al.* have also

the morphology of endothelial cells leading to pleomorphism and polymegethism.^{15,16} In this study, there were increased hexagonality values in healthy subjects than in uveitis patients. Our study found a negative effect of anterior chamber inflammation on corneal endothelial cell morphology. Similar findings were reported by Brooke *et al.* who suggested that heterochromic cyclitis causes changes in endothelial cell morphology, chiefly in older patients. They attributed it to the long period of hypoperfusion and intercellular oedema.¹⁷

Ozdamar *et al.* reported increased thickness in active uveitis in Behcet's disease than inactive and control Group.¹⁸ Cankaya *et al.* reported decreased CD and HEX in inactive period inpatient of Behcet's disease, but no difference in CCT was seen.¹⁹ Similarly, Guclu *et al.* has shown decreased CD in patients with

uveitis, but there was no difference in CCT1, whereas, in our study, we observed that there was a statistically significant difference between the Groups and patient with uveitis had increased corneal thickness (CCT) than healthy subjects. Finally, Phillai *et al.* has reported that there was the resolution of changes in endothelium once uveitis has settled,⁷ whereas, in our study, it was found that there is no difference between inactive and active uveitis.

There is a correlation between age and corneal endothelium and a decrease in CD hexagonality with age.² Glaucoma is described as one of the main complications of Fuch's uveitis,²⁰ but was not compared in this study. Coefficient of variation is a criterion of polymegathism that shows the variation between cell areas and is defined as the ratio of the standard deviation of cell areas in an endothelial zone to the average cell area.^{17,20} No statistically significant difference was seen between the Groups.

STUDY LIMITATIONS

Our study had some limitations, and we did not assess the relationship between intraocular pressure (IOP) and corneal endothelium in our study, although IOP can potentially influence the corneal endothelium. Analyzing the effect of IOP in these patients with uveitis could have given valuable information. Specular microscopy was performed only on one follow-up visit on every patient in this study, whereas performing it on multiple visits and comparing corneal endothelial markers could have given more precise data. This study did not assess the presence of keratic precipitates and their relationship to surrounding endothelium. This could have added beneficial information. The Duration of disease was also not considered uveitis patients.

CONCLUSION

In conclusion, notable changes are seen in central corneal thickness, endothelial cell density, cell size, and morphology in patients with uveitis compared to healthy subjects. In contrast, there was no difference in corneal endothelial markers in patients with active uveitis and inactive uveitis. Ophthalmologists should keep in mind these effects on endothelium uveitis patients and assess corneal health before any intra-ocular intervention to prevent further damage in such patients.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

QAG: Study design, drafting the manuscript, critical review, approval of the final version to be published.

OZ: Critical review, interpretation of data, approval of the final version to be published.

NNA: Data acquisition, data analysis, approval of the final version to be published.

MMA: Study design, Critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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