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ORIGINAL PAPER

Comparison of corneal endothelial cell analysis in patients with uveitis and healthy subjects

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Abstract
Purpose The aim of this study is to investigate the effect of uveitis in corneal endothelial cell number and morphology by non-contact specular microscopy.
Methods Our cross-sectional study was performed on 56 eyes of uveitis patients and 53 eyes of healthy subjects. Non-contact specular microscopy was performed to all subjects. The cell density (CD), coefficient of variation, cell minimum area (Min) and cell maximum area (Max), the average of cell size (AVG), percent of hexagonality (HEX%), central corneal thickness (CCT), intraocular pressure (IOP) during uveitis and during remission were measured and compared between two groups.
Results The mean endothelial cell analysis of the patients was 2540 ± 619 cells/mm^2, and the mean endothelial cell analysis of the control group was 2834 ± 413 cells/mm^2. The difference was statistically significant between the groups ($p = 0.01$). There was a statistically significant difference between two groups in terms of Max, Min, AVG, and HEX values. However, there was no difference in terms of CCT between two groups. There was a significant negative correlation between CD and IOP during uveitis attack. There was a significant negative correlation between the anterior chamber cell value and CD.
Conclusion Our results suggested that uveitis affected endothelial cell density, cell size and shape but not the corneal thickness without being influenced by the duration and number of attacks. Increased IOP during uveitis and anterior chamber cell value had an important role on CD in patients with uveitis.

Keywords Specular microscopy · Endothelial cell density · Coefficient of variation · The average of cell size · Percent of hexagonality

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Introduction

Corneal endothelium has an important role in corneal transparency and stability [1]. The metabolic activity of endothelium is essential for the stroma, which has been dehydrated for transparency [2]. Endothelium contains a monolayer of hexagonal cells with restricted regeneration ability [2, 3]. Endothelial cell density (CD), pleomorphism (characterizes the percentage of six-sided cells), polymegethism [cell size variability and it originates from coefficient of variation (CV)] are the markers of healthy cornea endothelium [4]. Aging, trauma, ocular surgical manipulation, diabetes, race, genetics, and gender are the causes of endothelial cell loss [5].

Uveitis can cause corneal alterations such as keratic precipitates, band keratopathy, corneal edema, iridocorneal attachments [6]. Intraocular chronic

inflammation induced from inflammatory cytokines in aqueous humor results with endothelial cell damage [7]. Most of the uveitis patients require intraocular surgery during their lifetime due to the complications. This situation increases the importance of endothelial function in uveitis patients.

Non-contact specular microscopy provides in vivo evaluation of the corneal endothelium and is constantly used in clinical practice [8]. Endothelial examination can be performed without anesthesia, and they do not require corneal contact therefore there is no risk of trauma or infection [9]. The TOPCON SP-2000P IMAGE-net semiautomated method is a non-contact specular microscope and allows high-quality endothelial images to be recorded readily [10]. It is easier to use, equipped with autofocus and built-in image analysis softwares [10]. An experienced examiner defines the measurement area manually [8]. The non-contact specular microscope captures the central region of the corneal endothelial image [8]. It enables automatic identification of the boundaries of corneal endothelial cells [8]. The manual clarification of the endothelial cell boundaries is the last step of the process [8]. Then the software calculates the endothelial parameters [10].

The aim of this study is to investigate the effect of uveitis on corneal endothelial cell number and morphology by specular microscopy and compare the endothelium of uveitis patients with age- and gender-matched healthy subjects.

Materials and methods

The present cross-sectional study is performed on 56 eyes of inactive anterior uveitis patients (22 idiopathic, 9 psoriatic arthritis, 13 Behcet disease, and 12 ankylosing spondylitis) and 53 eyes of healthy subjects at the Ophthalmology Department of Trakya University Faculty of Medicine. All procedures were performed in accordance with the ethical standards of the institutional committee and Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. The uveitis patients had to meet the following criteria for study inclusion: absence of any active inflammation in the eye within the last 6 months, uveitis was controlled with immunosuppressive treatment; after acute attack, the immunosuppressive treatment had continued; and there were no active uveitis symptoms. History of any ocular surgery, glaucoma, keratoconus, endothelial dystrophy, corneal scar, contact lens usage, and systemic diseases such as diabetes, kidney and liver diseases, malignancy, and smoking were the exclusion criteria. All patients underwent rheumatologic and infection examination and a full ophthalmologic examination including detailed medical history, best corrected visual acuity, slit lamp, intraocular pressure, fundoscopy, and specular microscopy. The interval since diagnosis of uveitis and number of uveitis attacks was also recorded. Only the affected eye of the uveitis patients is included in the study. The results are compared with the healthy subject group. For purposes of analysis, one eye of the bilateral uveitis patients was included to the study. For those patients with bilateral uveitis, we took into account the fact that decisions would always be based on the most affected eye [11]. The number of uveitis attacks was the criteria we used for selecting one eye from bilateral uveitis patients. However, if both eyes had the same attack number, the anterior chamber cell value was taken into consideration. The standardization of uveitis nomenclature (SUN) working group grading scheme for anterior chamber cell was used as previously described [12].

The number of cells in 1 mm^2 area defines endothelial CD. Hexagonality (HEX) means the percentage of six-sided cells which demonstrates pleomorphism. CV is calculated using the standard deviation (SD) of the mean cell area/Mean cell area (mm^2) ratio. Endothelial CD, HEX, CV, cell minimum area (Min) and cell maximum area (Max), the average of cell size (AVG) values were independently calculated in the center of the cornea by same experienced examiner with the Topcon SP-2000P (Topcon, Tokyo, Japan) specular microscope. Three digital images of central cornea were obtained, and three images averaged for the endothelial cell analysis. The examiner determined that the clearest image was captured, and the endothelial cell analysis was performed for the clearest images. Grading scale for the quality of images captured by the SP-2000P was used as previously described [1]. The procedures of the study were approved by the institutional review board of the hospital and adhered to the tenets of the Declaration of Helsinki.

Statistical analysis was performed using SPSS 20 (SPSS inc. Chicago, IL) to compare the effects of uveitis on corneal endothelial cells with healthy

subjects. The normal distribution was tested by the Kolmogorov–Smirnov test. The independent samples t-test was used for the comparison of the variables between groups. Evaluation of relations between measured variables was performed by Pearson's correlation. $P < 0.05$ value was considered as significance, for all analyses.

Results

The mean age of the patients was 46.21 ± 15.9 years in uveitis group and 46.42 ± 15.9 years in healthy subjects ($p = 0.948$). Thirty-six patients had unilateral uveitis, and twenty patients had bilateral uveitis. The mean interval since diagnosis of uveitis was 35.2 ± 36 months, and the mean number of uveitis attacks was 3.1 ± 2.3. The demographics and clinical characteristics of the patients and control group are shown in Table 1. There was statistically significant difference between the two groups in terms of Max, Min, AVG, CD, and HEX values (Table 2). There was no difference in terms of CCT between two groups (Fig. 1). There was no statistically significant difference between the unilateral and bilateral uveitis groups (Table 3).

In correlation analysis, there was a negative correlation between age and CD in control group ($r = -0.34$, $p = 0.01$). However, we found no correlation between age and CD in uveitis group. Also, we detected a significant negative correlation between CD and AVG in uveitis and control group ($r = -0.9$, $p = 0.00$, $r = -0.8$, $p = 0.001$).

There was no significant correlation between the duration of uveitis and Max, Min, CV, AVG, CD, and HEX values in uveitis patients. Similarly, we found no correlation between number of attacks and corneal endothelial cell values in uveitis group.

There was a significant positive correlation between Max, Min, AVG, CV values and CCT in uveitis group ($r = 0.2$, $p = 0.05$, $r = 0.3$, $p = 0.01$, $r = 0.2$ $p = 0.05$, $r = 0.5$ $p = 0.007$). In contrast, there was a negative correlation between age and CCT in uveitis group.

We did not detect pigmented or non-pigmented keratic precipitates in our patients group. All the uveitis patients had level 2 + to 4 + anterior chamber cells during the uveitis attack. Twenty-one patients had hypopyon. The IOP during uveitis was 15.7 ± 4.5 mmHg, and IOP during remission was 14.4 ± 2.7 mmHg. There was a statistically significant difference between the IOP during uveitis and remission ($p = 0.02$).

There was a significant negative correlation between CD and IOP during uveitis attack ($r = -0.68$, $p = 0.001$). We detected a significant correlation between IOP during uveitis and CV ($r = 0.29$, $p = 0.03$), and AVG ($r = 0.77$,

Table 1 Demographic and clinical characteristics of study patients and control group

Characteristic	Patient group ($n = 56$)	Control group ($n = 53$)
Age (years)		
Mean ± SD	46.21 ± 15.9	46.42 ± 15.9
Gender		
Male/female	29/27	28/25
IOP during remission (mmHg)		
Mean ± SD	14.4 ± 2.7	14.0 ± 2.8
IOP during acute attack (mmHg)		
Mean ± SD	15.7 ± 4.5	NA
Diagnosis		
Idiopathic	22	NA
Psoriatic arthritis	9	NA
Behcet disease	13	NA
Ankylosing Spondylitis	12	NA
Laterality		
Unilateral	36	NA
Bilateral	20	NA

IOP intraocular pressure, *SD* standard deviation, *n* eyes, *NA* not available

Table 2 Specular microscopy outcomes and statistical analysis between groups

Parameters	Uveitis group	Control group	p*
Max	1612 ± 186 μm^2	1203 ± 88 μm^2	0.05
Min	118 ± 71 μm^2	89 ± 56 μm^2	0.02
AVG	423 ± 132 μm^2	356 ± 64 μm^2	0.01
CD	2540 ± 619 cells/mm^2	2834 ± 413 cells/mm^2	0.01
HEX	49 ± 21%	57 ± 11	0.01
CV	38 ± 29	27 ± 16	0.02
CCT	522 ± 39	531 ± 39	0.21

Max cell maximum area, *Min* cell minimum area, *AVG* average cell size, *CD* cell density, *HEX* hexagonality, *CV* coefficient of variation, *CCT* central corneal thickness

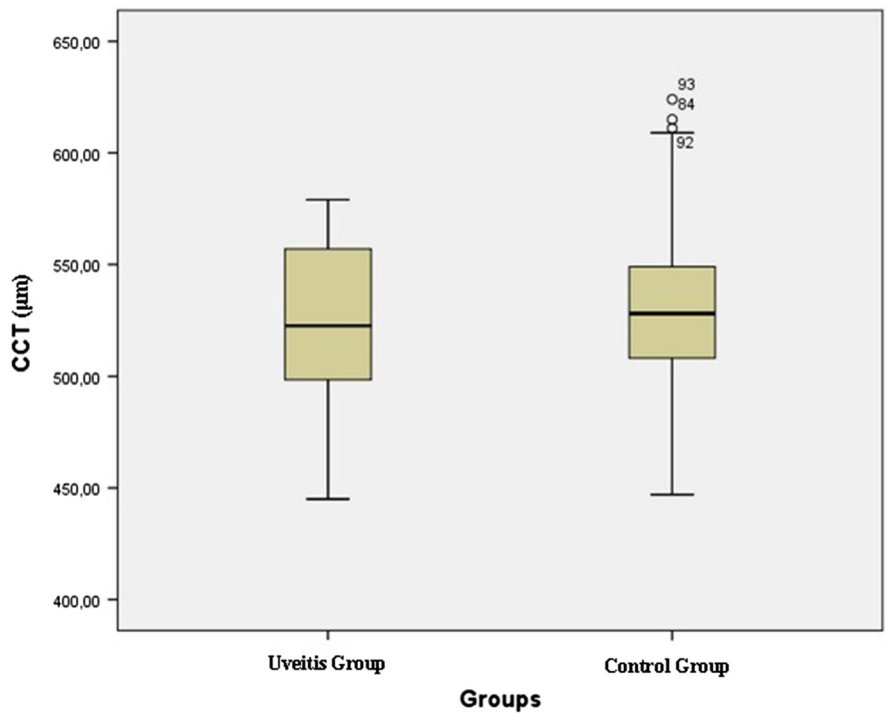

Fig. 1 Central corneal thickness of uveitis group and control group

$p = 0.001$). However, there was no correlation between IOP during uveitis and HEX ($r = 0.06$, $p = 0.61$). There was a significant correlation between IOP during remission and AVG ($r = 0.25$, $p = 0.05$), and CV ($r = 0.26$, $p = 0.04$). We did not find a correlation between CD and IOP during remission ($r = -0.1$, $p = 0.3$).

We found a significant negative correlation between the anterior chamber cell value and CD ($r = -0.77$, $p = 0.001$). There was a significant positive correlation between the anterior chamber cell value and AVG ($r = 0.78$, $p = 0.001$), IOP during uveitis ($r = 0.70$, $p = 0.001$), and IOP during remission($r = 0.28$, $p = 0.03$). However, we did not find a correlation between the anterior chamber cell value and CCT ($r = 0.18$, $p = 0.1$).

Discussion

In the present study, we found decreased CD and increased AVG in uveitis patients. The healthy

Table 3 Specular microscopy outcomes and statistical analysis between unilateral and bilateral uveitis group

Parameters	Unilateral uveitis group	Bilateral uveitis group	p^*
Max	1641 ± 248 μm^2	1560 ± 278 μm^2	0.84
Min	122.3 ± 79 μm^2	110.5 ± 56 μm^2	0.56
AVG	428 ± 125 μm^2	413 ± 146 μm^2	0.68
CD	2540 ± 517 cells/mm^2	2464 ± 779 cells/mm^2	0.50
HEX	49.06 ± 9.5	49.7 ± 14.1	0.84
CV	37.5 ± 7	39.2 ± 9	0.44
CCT	521 ± 34	522 ± 48	0.94

Max cell maximum area, *Min* cell minimum area, *AVG* average cell size, *CD* cell density, *HEX* hexagonality, *CV* coefficient of variation, *CCT* central corneal thickness

subjects had a decreased CV and increased HEX values than the uveitis patients. Our findings suggest that intraocular inflammation in the anterior chamber affects the corneal endothelial cell viability negatively in uveitis patients.

Endothelial cell analysis with non-contact specular microscopy plays an important role in the investigation of the strength and function of cornea [13]. Corneal transparency is provided by healthy cornea endothelium which pumps the water from the stroma through the aqueous humor and sustains a dry cornea with normal thickness [14, 15]. Corneal endothelial abnormalities in uveitis are reported in previous studies [6, 16–18]. Alfawaz et al. [6] suggested that unilateral uveitis patients had lower CD in the affected eye than the other eye without uveitis. In contrast, Olsen et al. [19] found that 15% of the unilateral uveitis patients had lower CD than the unaffected eye. These conflicting results are explained to be due to the long process of the disease. They have stressed that the chronicity of the disease has a negative correlation with CD [6]. However, in our study, we did not find a correlation between the duration of uveitis and the number of attacks and CD, because we did not use the unaffected eye of unilateral uveitis patients as a control group. Previous studies showed that corneal endothelium of the unaffected eye may be influenced by the inflammation in the body [6, 19]. Basarir et al. [20] demonstrated there was no significant difference between the affected and unaffected eye by the laser flare photometer in passive convalescent period of uveitis patients.

It was reported that the endothelium near keratic precipitates had larger cell size and decreased cell density than the normal endothelium [18]. We could not investigate the effect of keratic precipitates on the endothelium because none of our patients had acute attack during endothelial cell analysis. CD was also found lower in uveitis patients than the control group [6]. Additionally, other hypoxic or inflammatory situations such as smoking, diabetes, or cannabinoid usage have been reported to be related with lower CD [21–23]. In the present study, we found lower CD in uveitis patients than the healthy subjects.

It was reported that pleomorphism and polymegethism of the cornea endothelium represent that the cornea is under stressful situation [24, 25]. In these conditions, the uniform hexagonal shape of the normal endothelium disappears [25]. Previous study has reported decreased hexagonality compared in affected eye and has suggested that is a result of direct contact of endothelial cells and inflammatory cells or cytokines, in the aqueous humor. [6] Chronic hypoxia is recommended to decrease the percentage of hexagonal cells [26, 27]. Diabetes has been reported to cause decreased percentage of hexagonal cells and increased coefficient of variation [28–30]. Our findings are similar with the previous publications. We also find reduced percentage hexagonality in uveitis patients than the control group.

We also detected significantly higher CV value in uveitis patients. The CV value was 38 ± 29 in uveitis group and it was 27.16 in the control group. In contrast, a study by Olievera et al. [16] showed the CV value as 27.69 in non-infectious uveitis patients; this result may be attributable to small sample size of their study.

Central corneal thickness was not found different from the eyes with uveitis and uninvolved eye in patients with bilateral uveitis [6]. Recent studies demonstrated diabetic patients have thicker cornea than the controls [21]. It has been reported that chronic smoking has no significant effect on corneal thickness in multiple studies [13, 23]. In a study by Ozdamar et al. [31], they have demonstrated that patients with active Behcet uveitis have increased corneal thickness when compared to control group and inactive group. They have suggested that there is no permanent change in CCT in Behcet patients who had recurrent uveitis. Heinz et al. [32] found corneal thickness returned to normal after the inflammation reduced. We also found no significant difference in terms of CCT between two groups.

Tugal-Tutkun et al. [33] demonstrated the mean flare was significantly higher in eyes with Behcet disease both during in remission and acute attacks by using laser flare-cell photometry. Ladas et al. reported increased flare could persist in patients with history of uveitis even after the regression of anterior chamber cells [34].

Previous studies have reported decreased CD with increasing age in healthy subjects [35–37]. We also found similar results in control group. However, there was no correlation between age and CD in uveitis patients. This result could be related with the fact that the younger patients in the uveitis group had more severe, long-standing acute inflammation than the older patients [38].

De Juan-Marcos et al. [39] demonstrated that endothelial cell density is significantly decreased, and CV and HEX of cells are increased in pseudoexfoliative eyes, particularly when intraocular pressure is high. In a study by Bozkurt et al. [40], CD of pseudoexfoliative glaucoma eyes was found to be significantly lower than pseudoexfoliation syndrome eyes, but CV and HEX did not show any significant difference. Similarly, a recent study reported that CD was significantly lower and CV was significantly higher in pseudoexfoliative glaucoma group than in healthy subjects [41]. In the present study, we found a significant correlation between CD and IOP during uveitis. CD was lower, and AVG and CV were higher in patients with increased IOP during uveitis attack. However, there was no correlation between CD and IOP during remission.

Alfawaz et al. [6] demonstrated that the maximum flare was associated with lower central CD, while maximum anterior chamber inflammatory cell values were not. Anterior chamber cell value was related with lower CD in our study. However, the evaluation of anterior chamber cells is limited by clinical quantification.

We hypothesized that continuous ongoing inflammation in aqueous humor in patients with history of uveitis affects the corneal endothelium without being influenced by the total interval since diagnosis of uveitis and number of attacks. The limitation of this study is that we could not be able to investigate the flare in anterior chamber and we measured only the central ECD and CCT so we have no idea which part of the cornea is affected mostly by uveitis.

In conclusion, our results suggested that uveitis affected central ECD, cell size and shape permanently but not the CCT. Additionally, we detected that increased IOP and anterior chamber cell value had an important role on CD in patients with uveitis. This should be kept in mind to prevent corneal complications in uveitis patients during their lifetime and before intraocular surgery.

Compliance with ethical standards

Conflict of interest All authors certify that they have no conflict of interest. Author Hande GUCLU has no conflict of interest; Author Vuslat GURLU has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Trakya University Faculty of Medicine Ethics Committee) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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