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Corneal Endothelial Changes in Behçet's Patients with Inactive Ocular Involvement

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ABSTRACT

Purpose: The purpose of this article is to evaluate alterations in the corneal endothelial layer in Behçet's disease (BD) with inactive ocular involvement using specular microscopy.

Materials and Methods: Thirty-three eyes of 33 BD patients who had at least one anterior segment involvement and no active inflammation in the last 3 months were included in the study (group 1). Twenty-seven of the 33 BD patients had an anterior uveitis attack and six of them had a panuveitis (both anterior and posterior involvement) attack. Thirty-three eyes of 33 age- and sex-matched healthy subjects were enrolled in the control group (group 2). Corneal endothelial cell density (CD), coefficient of variation (CV), hexagonal cell ratio (HEX), and central corneal thickness (CCT) were measured using specular microscopy (Konan Medical, Nishinomiya, Japan), and the results were compared between groups.

Results: The mean CD was 2739 ± 164.18 cells/mm^2 in group 1 and 2922 ± 107.60 cells/mm^2 in group 2 (p = 0.001). The mean CV was 32.9 ± 4.76 in group 1 and 28.5 ± 3.06 in group 2 (p = 0.001). The mean HEX was 44.7 ± 6.51 in group 1 and 49.7 ± 6.10 in group 2 (p = 0.019). The mean CCT was 545.75 ± 40.89 µ in group 1 and 545.66 ± 30.09 µ in group 2 (p > 0.05).

Conclusions: Ocular attacks in our BD patients may have caused permanent changes in the corneal endothelial layer. However, these changes did not lead to corneal decompensation, but further studies are necessary to confirm these results.

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Inactive Behçet's disease; cornea; corneal endothelial morphology; specular microscopy

Introduction

Behçet's disease (BD) is a chronic, inflammatory, multisystem disease characterized by recurrent aphthous ulcers, vasculitis, and intraocular inflammation. The incidence of BD is high in Mediterranean and East Asian countries. Although the etiopathogenesis is not yet known, the relationship between HLA-B51 and BD confirms a genetic effect. Common histopathological lesions in all affected organ systems involve obstructive vasculitis. The main pathological findings of BD include partial congestion in the small vessels and fibrinoid degeneration resulting from lymph mononuclear cell accumulation around the vessels, swelling, or proliferation of endothelial cells.[1–5]

According to The International Study Group for BD, the diagnostic criteria of BD are recurrent oral ulcerations (three attacks in a year) and at least two of the following: recurrent genital ulcers, ocular lesions, skin lesions, and a positive skin pathergy test.[6]

Ocular involvement usually occurs 3–4 years after oral and genital involvement. Ocular involvement has been reported in 23–96% of cases in various studies. The most common ocular finding of BD is bilateral, recurrent, nongranulomatous iridocyclitis; although the most common form is anterior uveitis, characteristic findings of BD include obstructive and necrotizing retinal vasculitis.[7–9]

Anterior segment findings occur in only 6–15% of BD patients, and include keratic precipitates, posterior synechia, peripheral anterior synechia, cataract, episcleritis, filamentous keratitis, and corneal ulceration.[10]

While there have been many studies reporting the corneal parameters of different types of anterior uveitis, few studies have reported corneal parameters of BD. In the current study, we therefore evaluated the corneal endothelium by specular microscopy in BD patients with inactive ocular involvement, and discussed the possible mechanism underlying the involvement of the corneal endothelium.

Materials and methods

The study was performed prospectively at the Department of Ophthalmology of Inonu University Medical Faculty. The study group (group 1) was composed of 33 eyes of 33 BD patients (22–57 years of age) who had at least one anterior segment involvement and no active inflammation in the last 3 months. Thirty-three eyes of 33 age- and sex-matched healthy subjects were enrolled in the control group (group 2).

All BD patients with involvement of at least one anterior segment attack were followed up monthly during this period. After ruling out ocular inflammation in the anterior segment of the BD patients using slit lamp examinations, all topical medications were stopped. Patients who did not experience a new attack in the 3 months following the last attack were included in the study.

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None of the BD patients were treated with topical medications such as topical corticosteroids or cycloplegic agents. In group 1, seven patients were treated with oral colchicine, two with oral azathioprine, and one with oral cyclosporine. Six patients in group 1 also had posterior segment involvement in addition to anterior segment involvement (panuveitis). The specular microscopy results of these six panuveitis patients are shown in Table 1. The number of ocular attacks differed among the patients (range: 1–6). No patients had keratic precipitates during the study.

All study participants were Caucasian, and all gave informed consent before the study commenced, in adherence with the tenets of the Declaration of Helsinki. The study was approved by the Malatya Ethics Committee (Reference number: 2017/92).

A complete ophthalmic examination was performed by the same ophthalmologist, which included a visual acuity measurement, slit lamp examination, intraocular pressure measurement, and fundus examination.

After ruling out ocular attack, specular microscopy (Konan Medical) imaging was performed on the eyes of BD patients and healthy subjects by an investigator blinded to the patients record. Each eye was measured three times and the average values were recorded. Participants who had a systemic disease except for BD, previous ocular surgery or laser therapy, a history of a corneal disorder, trauma history, or glaucoma were excluded from the study. Patients with diabetes mellitus were also excluded from the study because corneal endothelium could be affected.[11] Corneal endothelial cell density (CD) (cells/mm^2), coefficient of variation (CV), hexagonal cell ratio (HEX), and central corneal thickness (CCT) were calculated automatically using the software bundled with the specular microscope (Figure 1 and Figure 2), and the results were compared between groups.

Statistical analysis

SSPS for Windows statistical software (ver. 17.0; SSPS Inc., Chicago, IL, USA) was used for the analysis. According to the power analysis, at least 33 participants were required in each group. The results are expressed as means ± standard deviation (SD). According to the Shapiro Wilk test, parametric test statistics were used because the data were normally distributed ($p > 0.05$). An independent sample t-test was used to compare the two groups. A value of $p < 0.05$ was considered statistically significant. Pearson's correlation test was used for the correlation analysis.

Table 1. Specular microscopic and demographic data of the patients with panuveitis.

	CD (cells/mm^2)	CV	H	CCT (μ)	NOA	DIP
Patient 1	2604.00	33.00	48.00	484.00	4	3
Patient 2	2703.00	30.00	49.00	547.00	3	4
Patient 3	2618.00	32.00	48.00	532.00	4	4
Patient 4	2439.00	28.00	43.00	593.00	5	4
Patient 5	2639.00	3700	42.00	561.00	4	3
Patient 6	2786.00	40.00	41.00	540.00	3	3

CD: endothelial cell density, CV: coefficient of variation, HEX: hexagonal cell ratio, CCT: central corneal thickness, NOA: number of attacks related to anterior segment, DIP: duration of inactive period (months)

Results

The mean age of the BD and control groups was 42.6 ± 9.35 and 40.2 ± 8.64 years, respectively ($p = 0.63$) (Table 2). The mean disease duration of the BD patients was 6.5 ± 5.2 years. The average best-corrected visual acuity was 0.13 ± 0.26 logarithm of the minimum angle of resolution (logMAR) for the BD group and 0 logMAR for the control group. The mean number of attacks was 2.69 ± 1.28 (range: 1–6) for the BD patients. The average duration of inactive ocular involvement (uveitis-free) was 3.84 ± 0.97 months (range: 3–6 months). Intraocular pressure and anterior segment biomicroscopy examinations were normal in both groups. Optic atrophy, ghost vessels, and diffuse atrophy and gliosis of the retina were detected in six patients with BD as a posterior segment finding.

The mean CD was 2739 ± 164.18 cells/mm^2 for group 1 and 2922 ± 107.60 cells/mm^2 for group 2. The mean CV was 32.9 ± 4.76 for group 1 and 28.5 ± 3.06 for group 2. The mean HEX was 44.7 ± 6.51 for group 1 and 49.7 ± 6.10 for group 2. The mean CCT was 545.75 ± 40.89 μ for group 1 and 545.66 ± 30.09 μ for group 2 (Table 3) (Figures 3–**6**).

There was a statistically significant decrease in the CD and HEX in the BD group when compared with the control group ($p = 0.001$; $p = 0.019$, respectively). The mean CV for the BD group was significantly greater when compared with the control group ($p = 0.001$). There was no statistical significance in CCT between the groups ($p = 0.99$).

Pearson's correlation analysis showed a strong negative correlation between the number of attacks and the CD ($p = 0.005$, $r = -0.472$). There was no significant correlation between the uveitis-free period and any specular microscopy parameter (all $p > 0.05$).

Discussion

Clinically, specular microscopy is usually used for visualization and morphological analysis of the corneal endothelium. Quantitative analysis of images from specular microscopy facilitates quantitation of the CD, cell shape, and size variations (%). CV, HEX, and CD are the most important endothelial parameters indexed by specular microscopy. Other parameters include the total area, the largest cell area, the smallest cell area, the average cell area, and the SD.[12–15]

CV is an objective criterion of polymegathism that indicates the variability between cell areas and is defined as the ratio of the SD of cell areas in an endothelial zone to the average cell area. The normal value should be < 0.30 (30%). HEX is the ratio of hexagonal cells to other cells with different geometrical shapes. Ideally, this ratio should be 100% and is 60–70% in a healthy endothelial layer. CD is the cell number/mm^2 and the normal value in an adult is approximately 2400 cells/mm^2. The CCT can also be measured by specular microscopy. The CCT can be used clinically as an indicator of endothelial functions, including pump and barrier functions.[15–18]

Previous experimental and human studies have reported that ocular inflammation can affect endothelial functions. MacDonald et al. reported that intraocular inflammation

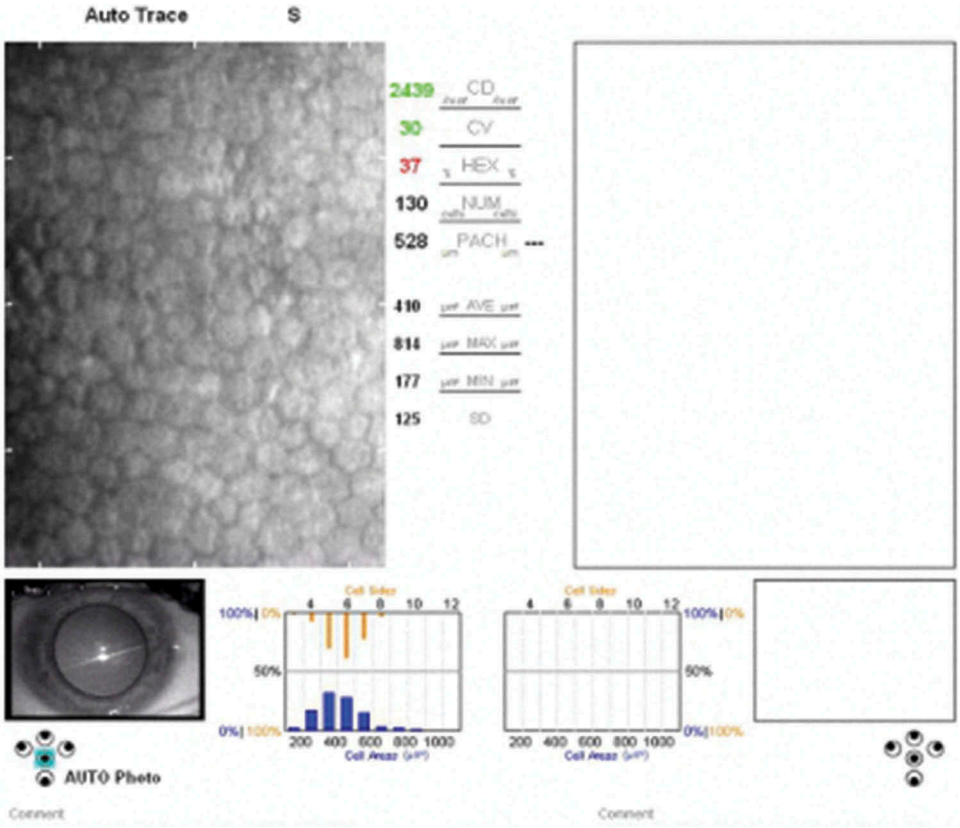

Figure 1. A shooting sample from group 1.

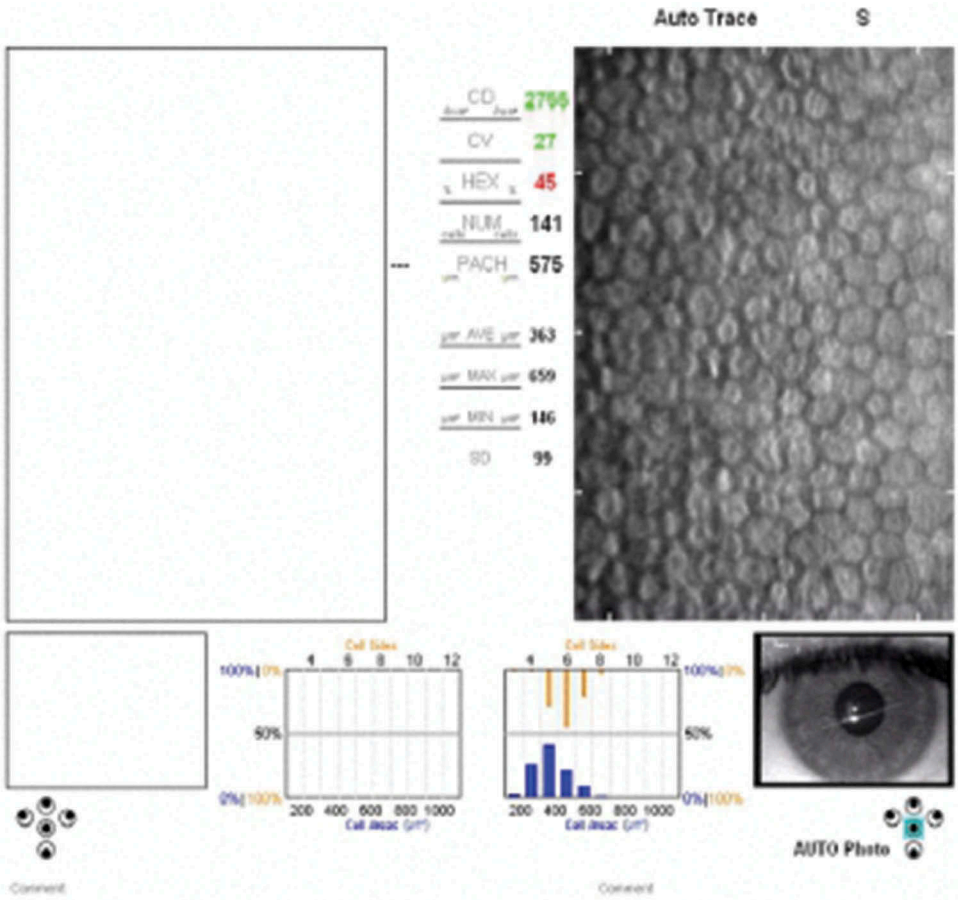

Figure 2. A shooting sample from group 2.

Table 2. Demographic features of the groups.

Features	Group 1	Group 2
Age, years (mean ± SD)	42.6 ± 9.35	40.2 ± 8.64
Gender (F/M)	15/18	16/17

F: female, M: male

Table 3. Mean CD, CV, HEX, and CCT values in group 1 and 2.

	Group 1 (mean ± SD)	Group 2 (mean ± SD)	*p* value
CD (cells/mm^2)	2739 ± 164.18	2922 ± 107.60	0.001*
CV	32.9 ± 4.76	28.5 ± 3.06	0.001*
H	44.7 ± 6.51	49.7 ± 6.10	0.019*
CCT (μ)	545.75 ± 40.89	545.66 ± 30.09	0.99

CD: endothelial cell density, CV: coefficient of variation, HEX: hexagonal cell ratio, CCT: central corneal thickness, *statistically significance

negatively affected corneal endothelial functions, resulting in an increase in corneal thickness.[19] Although there are numerous studies on the morphological and structural changes of the corneal endothelium during the acute phase of different types of uveitis[20], there have been few such studies in BD patients. Apart from inflammation, also other systemic diseases may affect the corneal endothelium. It has been revealed that higher HbA1c levels in diabetic patients were associated with lower CD and higher CCT values compared to healthy subjects. In the same study, it has also been revealed that good glycemic status had no impact on corneal endothelium.[11]

Banaee et al. reported an increase in the CCT in eyes with acute unilateral anterior uveitis when compared with unaffected eyes. However, in their study, no significant alteration was observed in affected eyes according to specular microscopy indices, and the CCT returned to the normal range within 1 week after treatment.[21] In contrast to their study, we found a lower CD and HEX, and higher CV, compared with the control group using specular microscopy.

Alfawaz et al. reported a lower CD in eyes with a history of uveitis compared with control eyes for all age groups. In the same study, the percentage of hexagonal cells was also lower in eyes with a history of uveitis compared with contralateral eyes. Furthermore, there was no significant difference in the CCT between eyes with and without uveitis.[22] Although the patient groups differed in terms of etiology (BD and uveitis without BD), our results were consistent with those of Alfawaz et al.

Szepessy et al. reported a decrease in the CD and CCT of patients with active Fuchs' uveitis syndrome compared with fellow eyes.[23] A common finding in uveitis without BD is that most eyes do not develop corneal decompensation, despite recurrent inflammation and endothelial abnormalities, and endothelial changes and corneal thickness return to normal values a few months after the attacks.

Recent studies have also reported that BD and other forms of endogenous uveitis differ in terms of the type of intraocular cytokines involved. Ahn et al. reported an extremely Th1-rich cytokine environment in Behçet's uveitis, whereas mixed Th1 and Th2 cytokines were found in the aqueous humor of other type of uveitis. In addition, proinflammatory cytokines were found in higher amounts in the aqueous humor of BD patients, while the levels of immunosuppressive cytokines were lower. Finally, natural killer cells, or CD8$^+$ T cell-activating cytokine and IL-15 ratios, in the aqueous humor were higher than in other types of uveitis, and TNF-α and IFN-ɣ levels were higher in the aqueous humor of BD patients.[24]

These results suggested a different immunopathogenic mechanism underlying the intraocular inflammation seen in BD patients. Behçet's uveitis may also cause more serious and devastating ocular injuries than other types of uveitis due to the presence of cytokines in different structures. Based on this, it is highly possible that the endothelial involvement in BD differs from that in other types of uveitis.

Both Ozdamar et al. and Evereklioglu et al. reported an increased CCT, which is an indicator of endothelial cell functions, during the active phase of BD.[25,26] However, during the

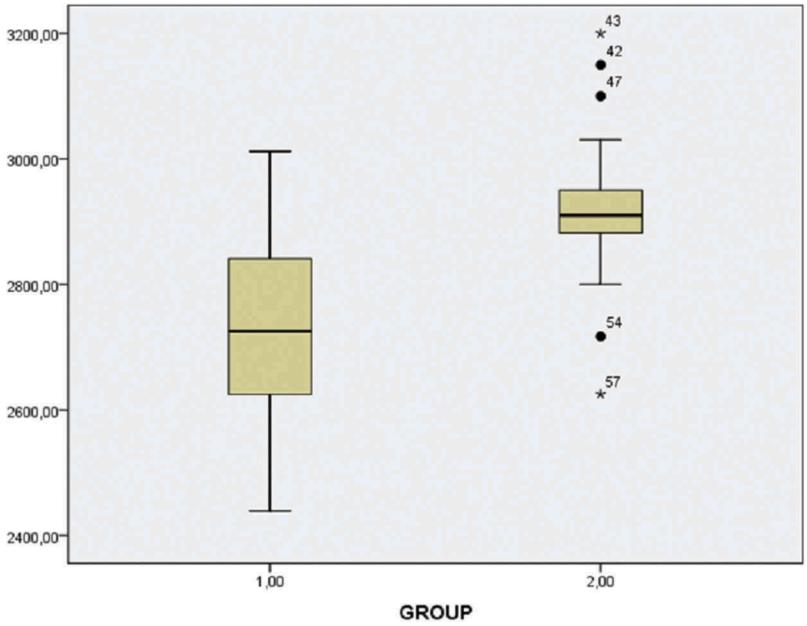

Figure 3. CD distribution according to groups.

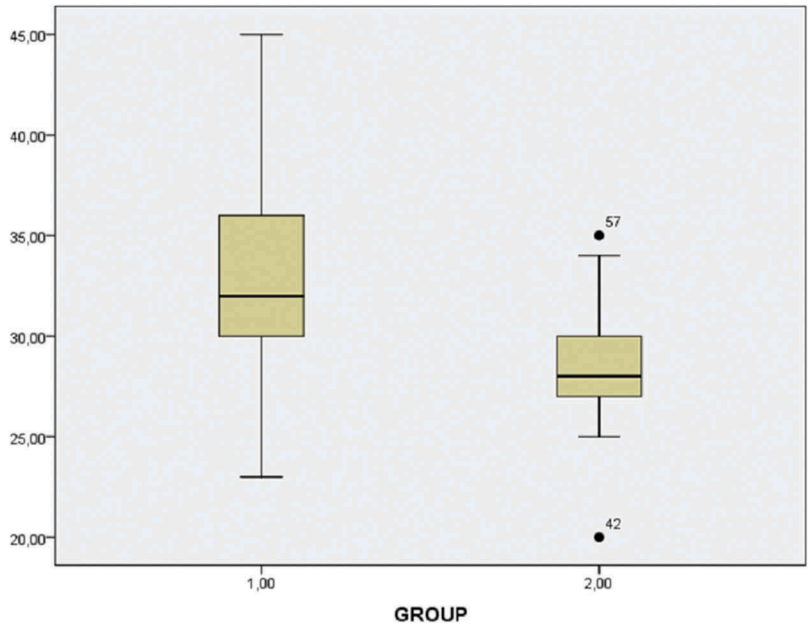

Figure 4. CV distribution according to groups.

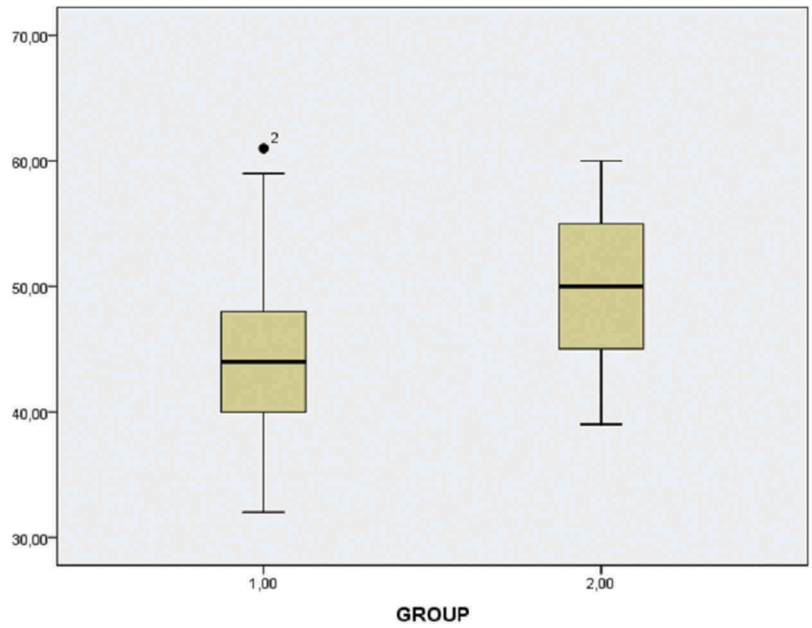

Figure 5. HEX distribution according to groups.

inactive period, no alteration was observed in the CCT. Cankaya et al. reported an increase in the CCT in an active BD group compared with inactive and control groups.[27] These findings are similar to our results, which revealed no significant CCT changes were observed in inactive BD patients. Taken together, the data showed a decrease in corneal endothelial functions during the active phase of BD. However, a normal CCT range during the inactive period suggested that the effect was temporary.

We hypothesize that not only chronic inflammation, but also oxidative stress impairs endothelial cell functions during both the active and inactive periods of BD. It has been reported that the oxidative stress-antioxidant defense balance is disturbed in favor of oxidative stress in BD. Excessive superoxide anion production, increased ADA activity (a marker indicating active neutrophil functions), hydrogen peroxide-induced hydroxyl radicals, and malonyl aldehyde production all indicate increased oxidative stress during BD. However, decreased superoxide dismutase, glutathione peroxidase, and catalase levels indicate a damaged antioxidant defense mechanism.[28] Several mediators and cytokines have been identified in the aqueous humor during anterior chamber inflammation, and have been reported to be associated with tissue damage and ocular complications. In addition to

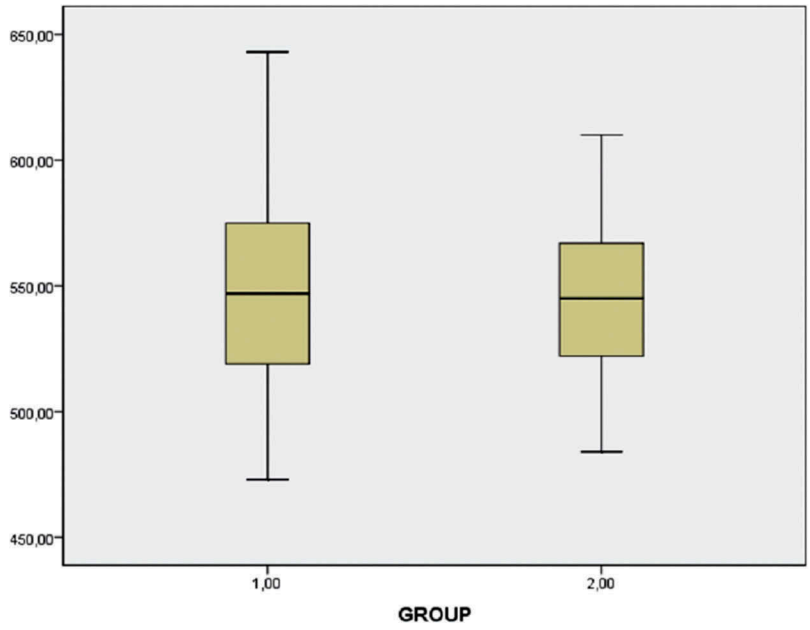

Figure 6. CCT distribution according to groups.

systemic cytokines, increased levels of other cytokines have been detected in the aqueous humor of BD patients.[29] Nitric oxide from free oxygen radicals has also been detected in the anterior chamber fluid and is thought to be the main stress mediator responsible for corneal endothelial cell damage.[30]

There were some limitations to our study. The most important limitation was that no specular microscopy data were obtained during the active period of BD; recording data during both the active and inactive periods of BD could have provided more valuable results. Another limitation was related to the systemic drugs used to treat BD patients. As mentioned in the Materials and Methods section, seven patients in group 1 were treated with oral colchicine, two with oral azathioprine, and one with oral cyclosporine. We could not find any study relevant to the direct effects of these medications on endothelial cell functions; however, they could have affected the disease activity and number and nature of the mediators and cytokines detected in the anterior chamber. In addition, six patients in group 1 had posterior segment involvement. We could not find any other report describing endothelial cell functions and posterior segment involvement during BD. However, we suggest that posterior segment involvement indicates disease activity. Regarding the use of specular microscopy, various sources of error may be present in all microscopes. To minimize these sources of error, all measurements were performed by the same operator and repeated three times, with the average values being recorded.

In conclusion, significant quantitative changes were observed in the CD, CV, and HEX using specular microscopy during inactive disease periods in our study. In particular, the correlation between CD and the number of attacks indicated that some endothelial cell damage occurred after each attack. However, it has been previously reported that such alterations in endothelial cells do not affect corneal decompensation. In general, our results are similar to other studies of non-Behçet's uveitis.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

1. Yazici H, Yurdakul S, Hamuryudan V. Behçet's syndrome. Curr Opin Rheumatol. 1999;11:53–57. doi:10.1097/00002281-199901000-00009.
2. Evereklioglu C. Ocular Behçet disease: current therapeutic approaches. Curr Opin Ophthalmol. 2011;22:508–16. doi:10.1097/ICU.0b013e32834bbe91.
3. Ebenezer AS, Moses DP, George R. Ocular manifestations of Behçet's disease. Indian Pediatrics. 2005;42:942–45.
4. Yazici H, Fresko I, Yurdakul S. Behçet's syndrome: disease manifestations, management and advances in treatment. Nat Clin Pract Rheumatol. 2007;3:148–55. doi:10.1038/ncprheum0436.
5. Evereklioglu C. Current concepts in the etiology and treatment of Behçet disease. Surv Ophthalmol. 2005;50:297–350. doi:10.1016/j.survophthal.2005.04.009.
6. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet. 1990;335:1078–80.
7. Ozdal PC, Ortac S, Taskıntuna I, Firat E. Posterior segment involvement in ocular Behçet's disease. Eur J Ophthalmol. 2002;12:424–31. doi:10.1177/112067210201200514.
8. Namba K, Goto H, Kaburaki T, Kitaichi N, Mizuki N, Asukata Y, Fujino Y, Meguro A, Sakamoto S, Shibuya E, et al. A major review: current aspects of ocular Behçet's disease in Japan. Ocul Immunol Inflamm. 2015;23:1–23. doi:10.3109/09273948.2014.981547.

9. Hatemi G, Seyahi E, Fresko I, Talarico R, Hamuryudan V. One year in review 2016: Behçet's syndrome. Clin Exp Rheumatol. 2016;34:10–22.
10. Ji YS, Yoon KC. A rare case of peripheral ulcerative keratitis associated with Behçet's disease. Int Ophthalmol. 2014;34:979–81. doi:10.1007/s10792-014-9910-3.
11. Storr-Paulsen A, Singh A, Jeppesen H, Norregaard JC, Thulesen J. Corneal endothelial morphology and central thickness in patients with type II diabetes mellitus. Acta Ophthalmol. 2014;92:158–60. doi:10.1111/aos.12064.
12. Huang J, Maram J, Tepelus TC, Modak C, Marion K, Sadda SR, Chopra V, Lee OL. Comparison of manual & automated analysis methods for corneal endothelial cell density measurements by specular microscopy. J Optom. 2017;7:1–10.
13. De Bernardo M, Rosa N. Comparison of specular microscopy and ultrasound pachymetry before and after cataract surgery. Graefes Arch Clin Exp Ophthalmol. 2017;255:837–38. doi:10.1007/s00417-017-3604-2.
14. Duman R, Tok Çevik M, Görkem Çevik S, Duman R, Perente I. Corneal endothelial cell density in healthy Caucasian population. Saudi J Ophthalmol. 2016;30:236–39. doi:10.1016/j.sjopt.2016.10.003.
15. Gasser L, Reinhard T, Böhringer D. Comparison of corneal endothelial cell measurements by two non-contact specular microscopes. BMC Ophthalmol. 2015;15:87. doi:10.1186/s12886-015-0068-1.
16. Mc Carey BE, Edelhauser HF, Lynn MJ. Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices, and new intraocular drugs and solutions. Cornea. 2008;27:1–16. doi:10.1097/ICO.0b013e31815892da.
17. Ohno K, Nelson LR, McLaren JW, Hodge DO, Bourne WM. Comparison of recording systems and analysis methods in specular microscopy. Cornea. 1999;18:416–23. doi:10.1097/00003226-199907000-00005.
18. Phillips C, Laing R, Richard Y. Specular microscopy. In: Krachmer JH, Mannis MJ, Holand EJ, Eds. Cornea Volume 1. 2nd. Philadelphia (PA): Elsevier Inc; 2005. p. 261.81.
19. Macdonald JM, Geroski DH, Edelhauser HF. Effect of inflammation on the corneal endothelial pump and barrier. Curr Eye Res. 1987;6:1125–32. doi:10.3109/02713688709034885.
20. Pillai CT, Dua HS, Azuara-Blanco A, Sarhan AR. Evaluation of corneal endothelium and keratic precipitates by specular microscopy in anterior uveitis. Br J Ophthalmol. 2000;84:1367–71. doi:10.1136/bjo.84.12.1367.
21. Banaee T, Shafiee M, Alizadeh R, Naseri MH. Changes in corneal thickness and specular microscopic indices in acute unilateral anterior uveitis. Ocul Immunol Inflamm. 2016;24:288–92. doi:10.3109/09273948.2014.970279.
22. Alfawaz AM, Holland GN, Yu F, Margolis MS, Giaconi JA, Aldave AJ. Corneal endothelium in patients with anterior uveitis. Ophthalmology. 2016;123:1637–45. doi:10.1016/j.ophtha.2016.04.036.
23. Szepessy Z, Toth G, Barsi A, Kranitz K, Naggy ZZ. Anterior segment characteristics of Fuchs uveitis syndrome. Ocul Immunol Inflamm. 2016;24:594–98. doi:10.3109/09273948.2015.1056810.
24. Ahn JK, Yu HG, Chung H, Park YG. Intraocular cytokine environment in active Behçet uveitis. Am J Ophthalmol. 2006;142:429–34. doi:10.1016/j.ajo.2006.04.016.
25. Ozdamar Y, Berker N, Ertugrul G, Gurlevik U, Karakaya J, Ozkan SS. Is there a change of corneal thickness in uveitis with Behçet disease? Cornea. 2010;29:1265–67. doi:10.1097/ICO.0b013e3181d142b3.
26. Evereklioglu C, Er H. Increased corneal thickness in active Behçet's disease. Eur J Ophthalmol. 2002;12:24–29. doi:10.1177/112067210201200105.
27. Cankaya C, Kalayci BN. Corneal biomechanical characteristics in patients with Behçet disease. Semin Ophthalmol. 2016;31:439–45.
28. Türsen Ü. Pathophysiology of the Behçet's disease. Patholog Res Int. 2012;2012:493015.
29. Yu HG, Lee DS, Seo JM, Ahn JK, Yu YS, Lee WJ, Chung H. The numbers of $CD8^+$ T cells and NKT cells increases in the aqueous humor of patients with Beçet's uveitis. Clin Exp Immunol. 2004;137:438–43. doi:10.1111/j.1365-2249.2004.02536.x.
30. Yilmaz G, Sizmaz S, Yilmaz ED, Duman S, Aydin P. Aqueous humor nitric oxide levels in patients with Behçet disease. Retina. 2002;22:330–35. doi:10.1097/00006982-200206000-00012.