In vivo confocal microscopy of corneal endothelium in patients with retinitis pigmentosa

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ABSTRACT

الأهداف: تقييم بطانية القرنية لدى المرضى المصابين بالتهاب الشبكية الصباغي وذلك باستخدام التنظير المبائر، ومقارنة نتائج هؤلاء المرضى مع مجموعة من الأصحاء.

الطريقة: أُجريت هذه الدراسة خلال الفترة من يونيو 2009م إلى يونيو 2011م وذلك في قسم العيون، جامعة سيلكوك الطبية، كونيا، تركيا. لقد قمنا بتقييم أعين 17 مريض مصاب بالتهاب الشبكية الصباغي مجموعة الدراسة1)، وأعين 17 فرد من الأصحاء مجموعة الشاهد2)، وقد كان للمجموعتين نفس العمر والجنس. ولقد قمنا بتقييم بطانية القرنية باستخدام التنظير المبائر، حيث قمنا بملاحظة كلاً من: كثافة الخلايا البطانية، وتغير حجمها، وتعدد أشكالها. وقمنا بالاستعانة باختبار مان ويتني من أجل مقارنة النتائج بين المجموعتين.

النتائج: لقد كانت متوسط القيم لدى المجموعة1 كالتالي: كثافة الخلايا البطانية 2440±451 خلية/مم² 1438–3037 خلية/مم²)، وتغير حجمها 48.3±8.3% 35.9–81.3%)، وتعدد شكلها 40.3±6.8% 24.7–53.8%. فيما كان متوسط هذه القيم لدى المجموعة2: كثافة الخلايا البطانية 2344±317خلية/مم² 1445–2903 خلية/مم²)، وتغير حجمها 45.3±9.2% 26.5–80.2%)، وتعدد شكلها 42.4±5.3% 24.8–49.5%. ولم يكن هنالك اختلافاً واضحاً من الناحية الإحصائية بين المجموعتين وذلك فيما يخص كثافة الخلايا البطانية p=0.15)، وتغير حجمها p=0.15)، وتعدد شكلها p=0.16).

خاتمة: أظهرت الدراسة بأن لم يكن هنالك فروق بين المرضى المصابين بالتهاب الشبكية الصباغي والأصحاء وذلك فيما يخص كثافة الخلايا البطانية، وتغير حجمها، وتعدد شكلها. وبما أن مرض التهاب الشبكية الصباغي يعد من مجموعة الأمراض المتباينة فنحن بحاجة إلى المزيد من الدراسات والتي تضم عدداً أكبر من مجموعات المرضى المتباينة.

Objectives: To evaluate corneal endothelium in patients with retinitis pigmentosa using confocal microscopy, and compare their results with those of healthy controls.

Methods: This comparative study took place between June 2009 and June 2011 in the Department of Ophthalmology, Selcuk University Faculty of Medicine, Konya, Turkey. We evaluated both eyes of 17 patients with retinitis pigmentosa (group 1), and the eyes of 17 age- and gender- matched control subjects (group 2). Corneal endothelium of all participants was evaluated using confocal microscopy (ConfoScan 3, Nidek Co. Ltd, Osaka, Japan). Endothelial cell density, polymegethism, and pleomorphism values were noted. Mann-Whitney U test was used to compare the results of these 2 groups.

Results: The values for mean endothelial cell density was 2440±451 cell/mm² (range: 1438-3037 cell/mm²), 48.3%±8.3 (range: 35.9-81.3%) for polymegethism, and 40.3%±6.8 (range 24.7-53.8%) for pleomorphism in group 1; while in group 2, these values were 2344±317 cell/mm² (range: 1445-2903 cell/mm²) for the mean endothelial cell density, 45.3%±9.2 (range: 26.5-80.2%) for polymegethism, and 42.4%±5.3 (range between 24.8-49.5%) for pleomorphism. No statistically significant difference was found between endothelial cell density (p=0.15), polymegethism (p=0.15), and pleomorphism (p=0.16) values of the 2 groups.

Conclusion: The endothelial cell density, polymegethism and pleomorphism values in patients with retinitis pigmentosa do not differ from those of healthy individuals. Since retinitis pigmentosa is a heterogeneous group of diseases, further studies with larger number and more homogeneous groups of patients are needed.

Retinitis pigmentosa (RP) is a heterogeneous group of disorder that cause progressive degeneration in the cone and rod photoreceptors, and in the retinal pigment epithelium (RPE).[1,2] The RP is the most common hereditary retinal degeneration worldwide. It presents with night blindness and loss of peripheral visual in adolescents and young adults. The major features of the disease include bone corpuscle-like pigmentation in the fundus, narrowing in the arterioles of the retina, waxy pallor of the optic disc, and electroretinography findings that signal the loss of photoreceptor function.[1,2] Although development of posterior subcapsular cataracts depends on the hereditary pattern, these cataracts are the most common anterior segment complication of RP.[2] Keratoconus is also more commonly observed in those with RP than in the normal population.[3] The bipolar horizontal cells of the inner nuclear zone, optic disc and vitreus, macula, crystalline lens, and the cornea may all be involved in the ocular manifestations of RP as well.[2] In addition, specular microscopy has shown corneal dystrophy, decompensation, and increased polymegethism and pleomorphism in those who have Kearns-Sayre syndrome, a syndromic form of RP.[4] In the literature, there is no controlled study, in which corneal endothelial cells was evaluated in RP. Since corneal endothelial cell count and morphology are determinants of a healthy cornea, the aim of this study is to evaluate the number and sizes of these cells in patients with RP using confocal microscopy.

Methods. This comparative observational study, which took place in the Department of Ophthalmology, Selcuk University Faculty of Medicine, Konya, Turkey between June 2009 and June 2011 adhered to the tenets of the Declaration of Helsinki, and the study protocol was approved by the local ethics committee. All patients gave their informed consent for the study. This study evaluated 68 eyes, the first 34 from 17 RP patients (Group 1) who had been referred to our ophthalmology clinics, and the remainder 34 eyes from 17 age- and gender- matched control patients (Group 2). The diagnosis of RP was based on both patient's history and clinical symptoms. The criteria used for inclusion in the study for all subjects (in both groups) were: having no more than a ±1 diopter of cylindric or spheric refraction error; had not been diagnosed

with diabetes, glaucoma, and ocular hypertension; and finally, had no signs of active or chronic uveitis. The exclusion criteria were: having been operated on for an eye disease; and having experience with laser applications; ocular trauma or contact lens usage; and having any corneal disease that might affect the cornea endothelium. All patients underwent detailed ophthalmic assessments, including visual acuity and intraocular pressure measurements, biomicroscopic anterior segment examinations and retinal evaluations. Confocal microscopy (ConfoScan 4.0, Nidek Co Ltd, Osaka, Japan) was performed by the same experienced technician, and under the same conditions (**Figures 1A, 1B, & 1C**). Topical anesthesia with 0.5% proparacaine was used before all measurements were obtained. The patients were positioned appropriately, and asked to stare at an internal fixation light. The objective (with 20X) was then brought closer to the eye that was being examined, without touching the cornea. After the levels of the corneal cells had been recognized, a scanner performed the examinations automatically. During the scanning process, the images can be viewed at the scanner monitor. Images of different levels of the cornea were recorded on the computer. From these images, the ratios between the endothelium intensity, pleomorphism, and polymegethism were calculated automatically.

The Statistical Package for Social Sciences version 16 (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. For both groups, the ratios between the cell intensity, pleomorphism, and polymegethism were compared using the Mann-Whitney U test, and a *p*-value lower than 0.05 was considered statistically significant.

Results. The mean age of the patients in Group 1 was 34.9±15.2 years (range: 14-73 years), and 46.4±12.3 years (range: 25-60 years) for Group 2. Both groups included 14 men and 3 women. In terms of age and gender, no statistically significant difference was found between the 2 groups (*p*=0.82, *p*=0.92 [95% confidence interval [CI]). When the confocal findings were compared, the intensity of the endothelial cells was 2440±451 cell/mm^2 in Group 1 patients, while in Group 2 it was 2344±317 cell/mm^2 (*p*=0.15 [95% CI]). The ratios of polymegethism in Group 1 was 48.3%±8.3 and 45.3%±9.2 for Group 2 (*p*=0.15, 95% CI). The ratios of pleomorphism were 40.3%±6.8 in Group 1, and 42.4%±5.3 in Group 2 (*p*=0.16 [95% CI]). There were no statistically significant differences in terms of endothelial cell counts, polymegethism ratios, or pleomorphism ratios between Group 1 and Group 2 (**Table 1**).
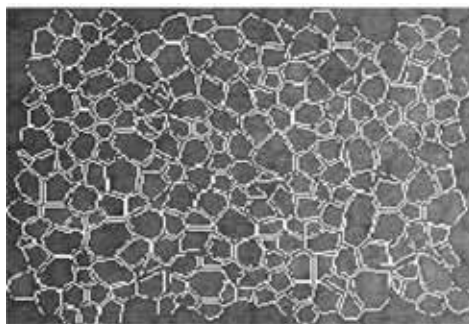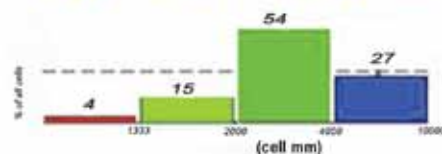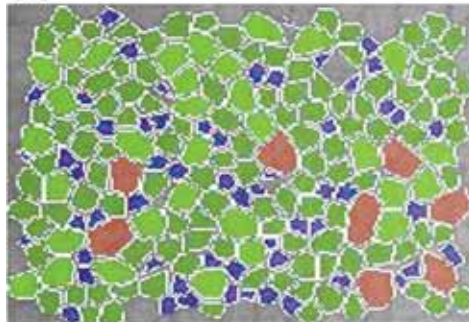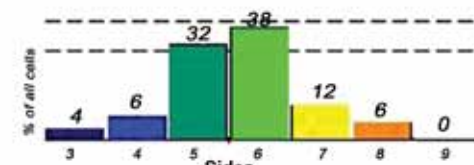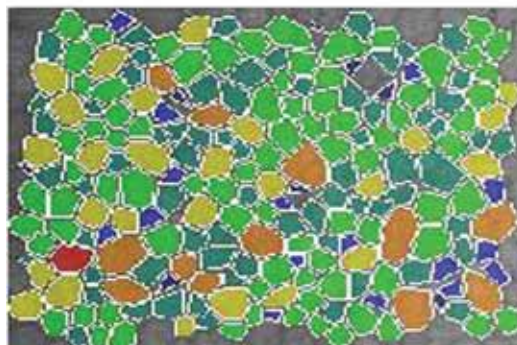
Figure 1 - A ConfoScan 4 of a patient with retinitis pigmentosa with normal cornea: **A)** endothelium; **B)** endothelial cell count and ratio of polymegethism; **C)** endothelial cell pleomorphism.

Table 1 - The confocal microscopy results of patients with retinitis pigmentosa (RP) and control patients included in a study conducted in the Department of Ophthalmology, Selcuk University Faculty of Medicine, Konya, Turkey.

Variables	Patients with RP (n=34)		Healthy subjects (n=34)		P (95% confidence interval)
	Median	Range	Median	Range	
Endothelial cell count (cell/mm^2)	2440	1438 -3037	2344	1445 - 2903	0.15
Pleomorphism (%)	40.3	24.7 - 53.8	42.4	24.8 - 49.5	0.16
Polymegethism (%)	48.3	35.9 - 81.3	45.3	26.5 - 80.2	0.15
p - significance value					

Discussion. The RP is a hereditary heterogenous group of disorders that cause degeneration in the rod and cone photoreceptors of the retina, and also affects the RPE.[1,2] Various chromosomal, metabolic, and morphological aberrations have been reported as causes of this progressive degeneration.[1-3] Today, no homogenous universal classification system exists for RP, however, it can be classified as primary (limited to the eye), and syndromic (involving one or more organ systems in addition to the eye). The RP can also be classified into subgroups, according to hereditary pattern.[2]

Although the frequency of developing posterior subcapsular cataracts depends on hereditary pattern, such cataracts are the most commonly seen anterior segment complication of RP.[2] Corneal endothelial cell loss is observed more often than would be expected after surgery in RP patients who have complicated cataracts. Because of this, it is important to evaluate the endothelial cell functions before cataract surgery in some of the patients with RP. Endothelial function can be evaluated in vivo, using confocal microscopy. This technique enables the analysis of endothelial cell intensity and morphology, including pleomorphism and polymegethism.[5]

Corneal endothelial cells do not show mitotic proliferation. When the number of endothelial cells decreases in some areas of the cornea, the sizes and shapes of the neighboring endothelial cells change, in order to cover this defect. As this occurs, the normal hexagonal shape of the corneal endothelial cells also change, and larger cells with different geometrical morphology are observed. Polymegethism occurs as a result of the increased size of the cells, and pleomorphism as a result of the different geometrical shapes.[6] While the increase in the ratio between the polymegethism and the pleomorphism shows corneal endothelial stress, the reduction in the intensity of

the endothelial cells is a sign of cell death.[6] When a corneal endothelial cell is affected, in cases like trauma, intraocular surgeries, chronic uveitis, acute angle glaucoma attacks, laser applications, diabetes mellitus and corneal endothelial dystrophies; the increase in the ratio between pleomorphism and polymegethism is observed first, followed by the decrease in the intensity of the endothelial cells.[6,7] Endothelial cell morphology may also change according to race, age, use of contact lenses, and myopia.[6]

In our study, no significant difference was observed in the pleomorphism and polymegethism ratios, and also in the endothelial cell intensity between patients with RP and the control patients. Although the mutations that cause the signs and symptoms of disease in RP, specifically affect the rod photoreceptors, the cone photoreceptors also become involved, in time.[8] Ripps[9] have shown that the rod photoreceptors secrete toxic metabolites, which affect the cone photoreceptors. Also, the RP may affect RPE and photoreceptor cells, as well as the bipolar and horizontal cells of the inner nuclear zone and the optic disc, vitreus, macula, crystalline lens, and cornea.[2] This supports the view that rod photoreceptors are initially affected, with other ocular manifestations seen over time. Oxidative injury provides another theory for explaining the death of cone photoreceptors after they become involved with the rod photoreceptors.[8] Since the formation of free radicals is one of the reasons for endothelium injury during phacoemulsification surgery, any mechanism that causes oxidative injury, such as in RP, may be the reason for endothelium damage.[10] Increased polymegethism and pleomorphism are reported in some syndromic RP patients, including those with Kearns-Sayre syndrome.[4] Since Kearns-Sayre syndrome evolves secondary to mitochondrial DNA deletions, the retinal pigment epithelium, corneal endothelium, and extraocular muscles are affected because of the high energy needs of these tissues.[4]

The sample size was the most prominent limitation in our study. Moreover, we have not grouped our study population according to mutation type, as no further genetic consultation could be performed in patients with a previous diagnosis of RP.

In conclusion, this study evaluated the corneal endothelium in patients with retinitis pigmentosa, and our study have found no statistically significant difference in the endothelium intensity, or in the ratios of polymegethism and pleomorphism between patients with RP and the control patients. Exclusion of syndromic patients from the study may have played a role on this result. However, since RP is a heterogenous group of disorders involving multiple mutations, further studies with homogenous and larger numbers of patients are needed.

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