Evaluation of the Effect of Topical Antiglaucoma Medications on Corneal Epithelium Thickness by Optical Coherence Tomography

Topikal Antiglokom İlaçların Kornea Epitel Kalınlığına Etkisinin Optik Koherens Tomografi ile Değerlendirilmesi

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ÖZ

Amaç: Topikal antiglokomatöz ilaçların (AGI) kornea epitel kalınlıklarına etkisinin araştırılması.

Gereç ve Yöntemler: Glokom tanısıyla takipte olan 58 hastanın retrospektif olarak optik koherens tomografiden elde edilen kornea epitel kalınlık haritaları, hasta grubunun kendi içinde ve glokomu olmayan 17 kontrol ile karşılaştırıldı. Gözler rastlantısal olarak seçildi.

Bulgular: Olguların ortalama yaşı 64.1±10.6 ve erkek/kadın oranı 34/41 idi. Ortalama yaş ve cins dağılımı hasta ve kontrol grubunda benzerdi. Hastaların, 33'ünde primer açık açılı glokomu,19'u psödoeksfoliasyon glokomu, 6'sında primer kapalı açı glokomu mevcuttu. Glokomu olan hastaların 15'ine yeni tanı konulmuştu ve AGI kullanımı yoktu. Yirmi sekiz hasta tek ilaç, 15 hasta ise birden fazla ilaç kullanmaktaydı. Kullanılan AGI sınıflandırıldığı zaman prostaglandin analoğu (PA) 13, PA da olan kombinasyon 18, PA dışı ilaç kullanımı 12 idi.

Yapılan ölçümlerde glokom grubu kontrolle karşılaştırılınca, kornea minimum kalınlık, superior (Esup), inferior (Einf), ve minimum epitel kalınlığı (Emin) daha düşük, epitel standart deviasyonu (Estd) daha yüksekti (p=0,027; p<0,001; p=0,003; p<0,001; p=0,045). AGI tedavisi alan hastalar, yeni tanı glokomlarla karşılaştırıldığında Esup, Einf, Emin daha düşük, Estd daha yüksekti (p=0,001; p=0,031; p=0,001; p=0,028). Birden fazla AGI kullanan hastalar tek ilaç kullananlarla karşılaştırıldığında Esup, Einf ve maksimum epitel kalınlık (Emax) daha düşüktü (p=0,028, p=0,020, p=0,028). Glokom tipi, AGI tipi ve kullanım süresi bakıldığında ölçümler arası fark yoktu.

Sonuç: AGI kullanımı kornea epitelinde incelmeye ve düzensizlikte artışa sebep olmaktadır ve bulgular birden fazla ilaç kullanımı ile artmaktadır.

Anahtar Sözcükler: glokom, kornea epitel kalınlığı, topikal antiglokomatöz ilaçlar.

ABSTRACT

Purpose: To investigate the effect of topical antiglaucoma medications (AGM) on corneal epithelial thickness (CET).

Materials and Methods: The CET measured by optical coherence tomography mapping of 58 patients has been compared within the study group and to 17 controls retrospectively. The eyes were selected randomly and operated eyes were excluded.

Results: Mean age of the participants was 64.1±10.6 years, and distribution of male/female ratio was 34/41. The mean age and gender distribution was similar in patient and control groups. The diagnosis was primary open angle glaucoma in 33, pseudoexfoliation glaucoma in 19 and angle-closure glaucoma in 6 patients. Fifteen patients had newly diagnosed glaucoma and receiving no AGM. Twenty-eight patients and 15 patients were under treatment with 1 and more than 1 drugs, respectively. AGM were prostaglandin analogue (PGA), combination with PGA and non-PGA in 13, 18 and 12 patients, respectively.

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The measurements in patients revealed that the corneal minimum, superior (Esup), inferior (Einf) and minimum epithelial (Emin) thicknesses were lower and standard deviation of epithelium (Estd) was higher than the control group. The patients under AGM treatment had lower Esup, Einf, Emin and higher Estd than the newly diagnosed cases. Patients who received multiple AGM had lower Esup, Einf ve maximum epithelial thickness (Emax) than the patients under single drug treatment. There was no difference in CET measurements regarding the glaucoma type, AGM type and medication duration.

Conclusion: Use of AGM causes thinning and irregularity of the corneal epithelium which is related with the number of drugs.

Key Words: glaucoma, corneal epithelium, thickness, antiglaucoma medications.

INTRODUCTION

Glaucoma is a progressive optic neuropathy with a characteristic optic disc cupping. It has an insidious course which may result in loss of vision. According to reports, glaucoma has been identified as the second leading cause of blindness worldwide.[1] Although management of the disease may require surgical intervention, topical medical treatment is the first choice for most of the patients. These topical anti-glaucoma medications (AGM) are mainly composed of beta-adrenergic blockers, alpha-adrenergic agonists, prostaglandin analogues (PGA), carbonic anhydrase inhibitors and cholinergic agents.

While these medications control the intraocular pressure (IOP), they may cause ocular surface irritation symptoms such as redness, burning sensation, itching and local side effects to the ocular surface, such as allergic conjunctivitis, dry eye, and pseudopemphigoid. Also, they may decrease the success rate of filtrating surgery, which has been well documented.[2] The toxicity of the AGM to the corneal epithelium was shown in cultured corneal epithelium in-vitro studies and in-vivo confocal examinations[3-6].

Most of these side effects develop due to the local inflammatory response of the cornea and conjunctiva to the medications. These side effects of the AGM may suggest structural changes to the corneal epithelium.

The present study was designed to investigate the effects of AGM on the physical property, the thickness of the corneal epithelium layer.

METHODS

This case-control study was conducted in 75 Caucasian participants between April 2015 and June 2015. The study group was composed of 58 glaucoma patients and 17 subjects constituted the control group. Patients with a history of ocular surgery, any other corneal disease, diabetes mellitus, and systemic connective tissue disease were excluded. Also, patients who wore contact lenses, used ophthalmic drops other than those with anti-glaucomatous properties, and those whose AGM regimen was changed within the last six months were also excluded.

Glaucoma was diagnosed by means of automated perimetry, optic disc appearance and IOP measurements. Pachymetry and corneal epithelial thickness measurements were performed by using optical coherence tomography (OCT, RTVue-XR, Optovue Inc., Fremont, CA). A non-contact anterior segment attachment lens (CAM-L) and automated image analysis system were used. Both scans were 6 mm. in diameter, centralizing the pupil with 8 radials, and 17 sectors. Software (version 5.5), automatically revealed an average epithelium thickness of 2-5 mm. superior and inferior sectors, the thinnest and thickest points and the standard deviations were Esup, Einf, Emin, Emax, and Estd (Figure 1, 2). The images were captured in a noncontact manner before the applanation tonometer measurements were taken by the same researcher. The measurements of a single randomly selected eye (38 right, 37 left) of each patient were included in order to obtain an independent comparison. Those patients who had previously undergone eye surgery, or who had other cornea diseases, were excluded. Three measurements were taken in micrometers (μm) and the one with the best signal quality was selected.

Statistical analysis was completed using the SPSS 20.0 software program. The comparison of proportions was done using the chi-square test, and the correlations were completed using the Pearson test. The continuous variables were compared through the use of a T-test, or by using the Mann-Whitney U test, where appropriate. A p value of less than 0.05 was selected to detect statistical significance.

Informed consent was obtained according to the Declaration of Helsinki. The study was approved by the local ethics committee (Approval: 23.03.2015-21/19).

RESULTS

The mean age of the participants was 64.1±10.6 years, and the male to female ratio was 34/41. The groups were similar regarding the mean ages (Mann-Whitney U test, p=0.06) and gender (chi-square test, p=0.134) distribution. The numbers of patients with primary open angle glaucoma (POAG), with pseudoexfoliation glaucoma (PEXG) and with angle-closure glaucoma (ACG) were 33, 19 and 6, respectively. Fifteen patients were newly diagnosed with glaucoma, and were not receiving medication, whereas 28 patients were on a single AGM and 15 patients were on multiple AGM. The distribution of patients for PGA, non-PGA and combination (PGA+non-PGA) therapies was 13, 12 and 18, respectively. Within the group of patients with glaucoma, 17 patients

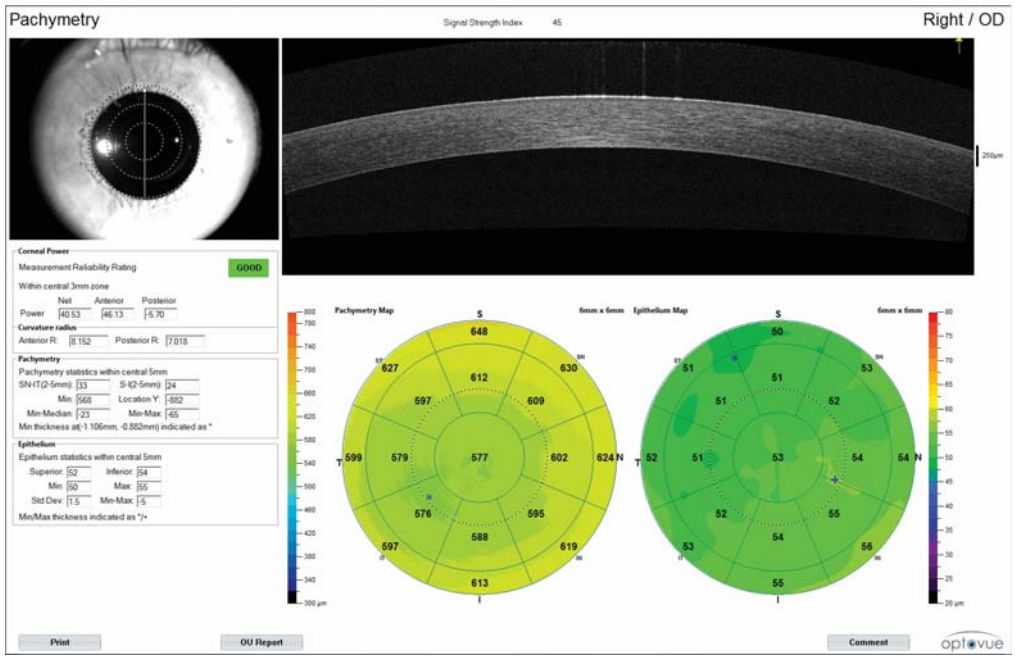

Figure 1. *OCT Pachymetry map of the corneal and epithelial thicknesses*

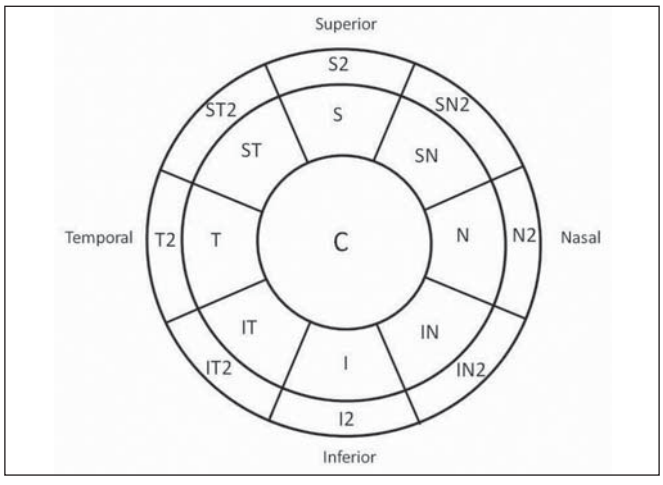

Figure 2: *Figure representing the sectors in 6 mm diameter of central cornea.*
(C: central, S: superior, I: inferior, N: nasal, T: temporal, SN: superonasal, ST: superotemporal, IN: inferonasal, IT: inferotemporal, S2, I2, N2, T2, SN2, ST2, IN2, IT2 are all peripheral components of the mentioned sectors.)

had been using medication less than two years (6 months-2 years) and 26 patients had been using medication more than two years (2-17 years).

OCT mapping revealed that in the patients with glaucoma, the minimum corneal (Cmin), Esup, Einf and Emin were less and Estd was greater than those of patients in the control group (Table 1). In patients with glaucoma who were on medication, Esup, Einf and Emin were less and Estd was greater than the newly diagnosed patients who were not using medication (Table 1). In patients whose medication consisted of more than 1 drug, Esup, Einf and Emax were less than those in the patients using a single drug (Table 1).

When we made similar comparisons with respect to all the corneal sectors separately, we detected that corneal thickness was less in the N, N2, IN, IN2, I, I2, IT and IT2 sectors of the glaucoma patients than of those in the control g, the epithelial thickness of all sectors of the glaucoma patients was less than those of the control group (Figure 2). When newly diagnosed glaucoma patients who had not received treatment were compared to the control group in terms of sectors, the corneal and epithelial thicknesses were similar.

The corneal and epithelial thicknesses of patients on medication were similar regarding the type of the drug (Mann-Whitney U test, $p>0.05$ for all measurements). There was no correlation between corneal or epithelial thickness measurements and AGM duration (Pearson correlation test, $p>0.05$ for all measurements).

Regarding the glaucoma type, the central corneal thickness (CCT) was greatest in the ACG patients than in the POAG and PEXG patients (Mann-Whitney test, $p=0.007$ for ACG vs PAAG, $p= 0.006$ for ACG vs PEXG and $p= 0.834$, respectively). However, there was no significant difference regarding the epithelial thicknesses of these 3 groups.

DISCUSSION

There are three important points regarding the cornea in glaucoma patients. First of all, it is the place where the IOP is measured and its irregularity and thickness may change the results, therefore corneal thickness needs to be estimated.[7] Secondly, CCT is an independent risk factor for glaucoma progression.[8] Finally, the cornea is the barrier that the AGM must pass through, and the potential surface where the drugs side effects may be observed. The corneal epithelium, the outer layer of the cornea, has non-keratinised stratified

Table 1. *Comparison of OCT mapping measurements within the study group and to healthy controls.*

	G(+) n: 58	G(-) n:17	P**	G(+)D(+) n: 43	G(+)D(-) n: 15	P**	G(+)D>1 n: 15	G(+)D=1 n: 28	P**
Cmin*	517(456-580)	531 (486-603)	0.027	-	-	-	-	-	-
Esup*	47 (40-54)	51 (46-56)	<0.001	46 (40-54)	50 (46-54)	0.001	44 (40-49)	47 (40-54)	0.028
Einf*	50 (41-57)	54 (42-54)	0.003	49 (41-57)	52 (48-56)	0.031	48 (41-52)	50 (45-57)	0.020
Emin*	43 (24-52)	47 (42-54)	<0.001	43 (24-52)	47 (42-52)	0.001	-	-	-
Estd*	2.15 (0.8-7.7)	1.7 (1-4.9)	0.045	2.5 (1.1-7.7)	1.9 (0.8-3.1)	0.028	-	-	-
Emax*	-	-	-	-	-	-	51 (44-60)	55 (47-66)	0.028

G: glaucoma, D: number of AGM
*: Measurements are given as median (minimum-maximum) in µm.
**: Mann-Whitney U test

cells, composed of 1 layer of basal cells, 2-3 layers of wing cells and 5-6 layers of surface flat cells. Normally all cells end up at approximately 50 µm.[9, 10] The cornea acts as a barrier against the environmental irritants and topical drugs as well.[9]

Although the toxic effect of the AGM was shown in histologic studies in human conjunctiva epithelium, it was demonstrated that there was an increase in inflammatory cells, a decrease in goblet cells, and an increase in apoptosis.[11, 12] There is a lack of In-vivo histopathologic studies on human cornea epithelium which examine the effects of AGM. A previous study compared the central CET measurements of 23 glaucoma patients with Sjögren'syndrome patients and controls.[13] Although they found no difference in the measurements, their study has shortcomings because only manual measurements were used, only the central CET was considered, and no distinction was made concerning the AGM types. A study on AGM using a 3D-reconstructed cornea epithelial model demonstrated decreased cell viability, increased apoptosis, tight junction loss, and increased inflammatory markers in direct proportion to an increase of AGM and preservative solution (Benzalkonium chloride: BAC) concentrations.[14] In another experimental study, the toxicity of AGM to the corneal epithelium was demonstrated, and it was postulated that a lower BAC concentration, or AGM without preservatives, were the least toxic.[3] In another in-vitro study on 10 rabbit eyes in which timolol with BAC was applied, researchers observed a decrease in CET, and proposed that it was the first sign of toxicity.[4] However, there is a concern whether these models really test the effects of long duration of usage, repeated use, and in vivo clearance of the drug by tears.

Agahian et al showed that in all types of glaucoma, the CCT was less than that of non-glaucomatous eyes.[15] Our analysis comparing the glaucoma patients (including both newly diagnosed and under treatment patients) revealed that the Cmin was less r than that of the control group. There was an interesting finding in comparison regarding the sectors: there was no difference in the CCT, but there was significant corneal thinness in the N, N2, IN, IN2, I, I2, IT and IT2 sectors of glaucoma patients than in the control group, where the duration of using eye drops on the ocular surface is prolonged (Table 2). Also, in all of the sectors, the corneal epithelium were thinner.

Medications used for glaucoma treatment may affect the corneal and epithelial thickness. Schrems et al. reported de-

Table 2. *Comparison of pachymetric measurements in sectoral basis between control and patient groups (only the significantly different sectors are given).*

Sectoral thickness*	Control (n= 17)	Patient (n=58)	P**
N	560±33	540±30	0.021
N2	582±32	561±33	0.024
IN	555±33	534±30	0.012
IN2	576±32	553±31	0.008
I	551±31	530±29	0.012
I2	571±30	549±30	0.011
IT	544±31	523±32	0.018
IT2	560±30	540±29	0.014

*: Measurements are given in µm with mean±standard deviation.
**: Independent sample T test

creased CCT in patients who were receiving PG, a PG combination, carbonic anhydrase inhibitors and beta-adrenergic blockers. Viswanathan reported similar results, except regarding the beta-adrenergic blockers.[16, 17] PGA in particular was believed to be causing the stromal collagen gel contraction, and altering the architecture.[18, 19] PGAs induce expression of matrix metalloproteinases in ciliary body.[20] However, in our cohort, within the medication receiving group, the type of the medication did not appear to have an effect on the thickness measurements of cornea and epithelium.

The CCT varies according to the glaucoma type, as it was shown to be less in open angle glaucoma when compared to ACG.[21] Our results also revealed that the CCT was greatest in ACG.

Our analysis comparing newly diagnosed patients with medication receiving groups revealed that the medication receiving group had lower Esup, Einf and Emin thickness; interestingly, Esup, Einf and Emax thickness was less in patients receiving more than 1 drug when compared to single drug users. These findings may be attributed to the local effects of the AGM, and the number of drugs, as the difference becomes prominent in patients who are using more than 1 drug. As the number of drugs increases, the preservative amount reaching the cornea epithelium also increases. Supporting these findings, Dogan et al. reported increased apoptosis rates in the conjunctival epithelial layer of glaucoma patients when compared to those patients in the controlgroups.[11] They claimed that the preservatives in the topical drugs was a causative factor. This is also valid for our patients. The effect of different preservatives on corneal epithelium may be another significant issue. However, all the medications included preservatives, albeit with different concentrations and combinations of drugs. Therefore, it was not possible to make a statistical analysis in order to evaluate this potential factor.

The corneal epithelium can also be evaluated through the use of confocal microscopy. To the best of our knowledge, there are two studies in the literature on the confocal microscopic evaluation of the corneas of glaucoma patients using AGM. In one of these studies, the authors showed that there was a decrease in the number of superficial cell counts, and there was an increase in the density of basal epithelial cells. Interestingly, they didn't find a decrease in superficial layer counts in glaucoma patients using preservative free drugs, which also supports our hypothesis.[5] They also detected that in all AGM users, subepithelial nerves were lower and tortuous compared to those of the control groups. This is similar to the confocal microscopic study of Mastropasqua et al. which demonstrated irregularity in the limbal transition epithelium.[6] We also found that in glaucoma patients and in drug using patients, Estd was higher, which may confirm this irregularity. The endothelial counts does also matter for stromal and eventually epithelial thicknesses. However, our study did not include confocal microscopy findings, and we are not able to comment on this issue. This can be the subject for a future study.

CET measurements require high resolution techniques. Besides OCT, these measurements can be made through the use of high-frequency scanning ultrasound biomicroscopy (HF-UBM), as well as confocal microscopy through focusing.[4, 22] However, these two latter techniques depend on a contact method, which may include operator-dependent factors, be uncomfortable for the patients, and be difficult to screen. OCT seems objective in terms of thickness measurements. The reproducibility of the results has been determined by a study which demonstrated repeatability with a 1.78±0.78 µm topographic variability.[10] Most of the previous studies examined the total corneal thickness. However, our study is the first study to investigate the corneal epithelial thickness, in AGM using group through OCT mapping, and to detect changes in this level. Our findings have important results which can affect the daily practice of glaucoma patients. These results can affect the use and the number of AGM, as these medications may cause irregularity and decreased thickness of the corneal epithelium.

There are some limitations of our study. Although we attempted to place the similar drugs in groups, because of the wide variety of AGM, it would be better to increase the number of patients. The cross-sectional nature of the study precludes interpreting the course of the measurements within time. Therefore, a study in newly diagnosed patients with a longer follow-up in order to observe the effects of the AGM would be elucidative.

CONCLUSIONS

Our results by means of OCT mapping showed irregularity and decreased thickness of corneal epithelium in glaucoma patients. The use and number of topical anti-glaucomatous drugs not the duration and type of AGM were found as determinant factors in this difference. This can be accepted as a drug toxicity. Therefore, we recommend that patients use as few drugs as possible, and that glaucoma patients receive regular follow-up through the use of non-contact OCT.

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