Evaluation of Corneal Transparency in Eyes Treated with Brimonidine

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ABSTRACT

Purpose: The aim of this study was to evaluate the effects of topical 0.1% brimonidine on the corneal densitometry.

Materials and Methods: This prospective study included 32 eyes of 17 patients who were diagnosed with primary open angle glaucoma and treated with 0.1% brimonidine, and 28 eyes of 14 healthy individuals. Corneal densitometry and corneal thickness (CT) were evaluated in the three corneal zones (0-2, 2-6, 6-10) by Scheimpflug corneal topography at baseline and on day 3, 17 and 30 after the treatment.

Results: The CT values in the central and zones of 2, 6 and 10 mm on day 3 after treatment were statistically significantly thicker when compared to baseline and controls ($p < 0.001$ for all). Corneal densitometry values in the central corneal layer in all three zones (0-2, 2-6, 6-10) on day 3 after treatment were statistically significantly higher when compared to baseline and controls ($p < 0.05$ for all). However, there was no significant difference between CT and corneal densitometry values obtained at baseline and day 7 after treatment or day ($p > 0.05$, for all).

Conclusion: Our results show that topical 0.1% brimonidine administration caused an increase in corneal thickness in all zones while an increase in corneal densitometry values in the central corneal layer in all three corneal zones at short-term. The temporary effect of brimonidine on the cornea is probably due to fluid accumulation in the central corneal layer.

Keywords: Brimonidine, corneal densitometry, corneal thickness.

INTRODUCTION

Brimonidine is a selective α-2 adrenoreceptor agonist used in the treatment of glaucoma and ocular hypertension, which is a safe and well-tolerated anti-glaucomatous agent.[1] It is rapidly absorbed by cornea and conjunctiva following topical administration and exerts its effect on ciliary body by suppressing humor aqueous production and increasing uveascleral absorption, presumably due to reduced blood flow.[2] In addition to ocular hypotensive effect on ciliary body, corneal and retinal effects are also reported.[3, 4]

Corneal transparency is highly important for visual function and can be quantified by measuring retrograde scattering of light from cornea. The structural alterations of cornea may reduced corneal transparency by influencing of light transmission and lead higher rates of retrograde scattering.[5] Cornea densitometry is a parameter of corneal transparency, allowing quantitative assessment of light scattering. Pentacam HR (Oculus Inc., Wetzler, Germany), is a non-contact, rapid, reproducible optical system using a rotator Scheimpflug camera. It allows assessment of anterior segment from corneal surface to posterior surface of lens, providing data about corneal densitometry which provides important data about corneal transparency.

Although there are studies investigating brimonidine on corneal thickness, there is limited data about structural changes in cornea.[4] In this study, it was aimed to assess short-term effects of topical brimonidine 0.1% on corneal densitometry given that changes occurring in patients treated with brimonidine 0.1% may affect corneal transparency.

MATERIALS AND METHODS

This prospective, observational study was conducted at Ophthalmology Department of Kütahya Health Sciences

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University between January, 2019 and May, 2019. The study was approved by Teaching And Planning Committee. The study was conducted in accordance to tenets of Helsinki Declaration. All were given written informed consent before study. The study included 32 eyes of 17 patients who were diagnosed with primary open angle glaucoma (POAG) and treated with 0.1% brimonidine (Alphagan P; Allergan, Irvine, CA), and 28 eyes of 14 healthy individuals. The study included the patients who could not tolerate ocular adverse effects of prostaglandin analogs and received topical brimonidine as first-line treatment. All subjects underwent a comprehensive ophthalmological examination including corrected visual acuity (VA) by Snellen charts, slit lamp and fundus examinations, intraocular pressure (IOP) measurement by Goldmann applanation tonometry, Humphrey visual field testing (30-2 program), retinal nerve fiber layer (RNFL) analysis by spectral domain-optical coherence tomography (SD-OCT) and corneal thickness (CT) and corneal densitometry (CD) measurements by Scheimpflug corneal topography. The VA, IOP measurement and corneal topography imaging were repeated on day 3, 7 and 30 after treatment. Based on ophthalmological examination. The patients aged 18-60 years fulfilling POAG criteria were included to the study. The patients previously received anti-glaucoma therapy, those with any diagnosis of glaucoma other than POAG (pseudo-exfoliative and pigmentary glaucoma), Grade <2 eyes by Shaffer grading system, those with history of ocular trauma, ocular surface disease or surgery, eyes with refractive error >3 diopter spherical or >1 diopter cylindrical were excluded. The patients with uncontrolled systemic disease that may affect corneal transparency, those with diabetes and those with collagen tissue disorders were also excluded.

Scheimpflug corneal topography uses a rotator camera to obtain 2-dimensional optical sections from cornea. The Pentacam system reformates 2-dimensional images of anterior and posterior corneal surface, providing 3-dimensional images. The corneal densitometry software estimates corneal densitometry value over a certain area (12 mm in diameter) by measuring retrograde light scattering from cornea. The densitometry value measured is expressed as grey tone units and numerical values (0, maximum transparency and 100, total opacity). The system makes the calculations separately for anterior corneal layer (120 µm in size), posterior corneal layer (60 µm in size) and the layer between anterior and posterior corneal layers at 4 apex-centered concentric zones (0-2, 2-6, 6-10 and 10-12 mm).[6] In all patients, cornea densitometry measurements at same time of the day (10:00-12:00) under standard light conditions by same experienced ophthalmologist (O.A). To minimize acquisition errors, we included high-quality

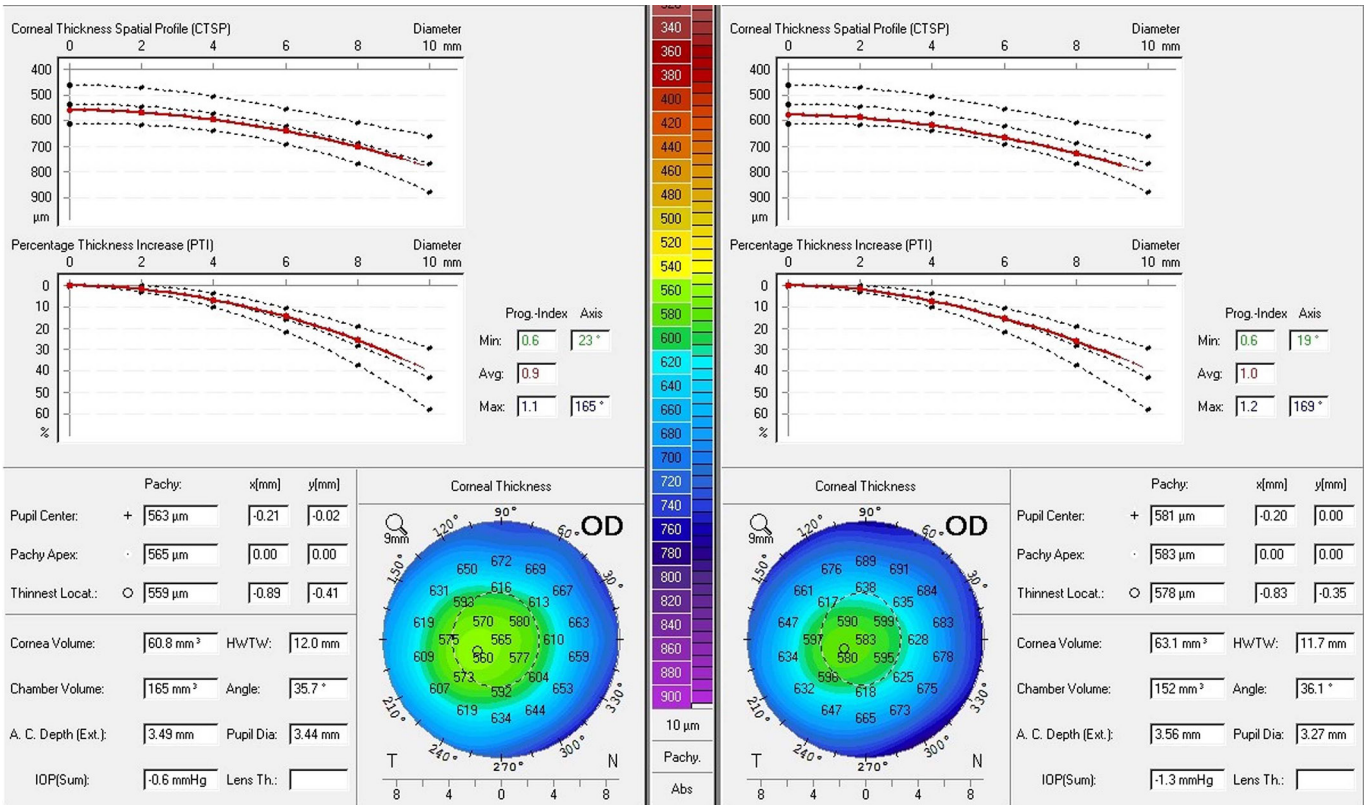

Figure 1:

Pentacam images which were labeled as "OK" on Quality Specification (QS) domain by Pentacam. Images with poor quality due to incompliance (2 eyes) were excluded. Since outermost zone (10-12 mm) showed lowest reliability and reproducibility, it was excluded.[6, 7] We prescribed topical brimonidine tartrate 0.1% preparation twice daily (Alphagan-P Allergan, Irvine, CA). Since fluorescein drop used for measurement by Goldmann applanation tonometry may affect light scattering, tonometry was performed after corneal topography imaging.

In the study, central corneal thickness was measured at baseline and after treatment in 3 corneal circles (2, 6 and 10 mm in size). The Pentacam device measures corneal thickness as the distance between anterior and posterior margins of cornea in whole area; thus, it defines the thinnest corneal point in all corneal circles and calculates mean corneal thickness in concentric circles.[8]

All statistical analyses were performed using SPSS for Windows version 21.0 (SPSS Inc., Chicago, Illinois). Continuous variables are expressed as mean±standard deviation. The normal distribution of data was assessed using Shapiro-Wilk test. The Dependent t test was used to compare pretreatment and post-treatment changes in continuous variables. The Independent samples t test was used to compare corneal values between patient and control groups. A p value <0.05 was considered as statistically significant. Pearson's correlation analysis was used to assess relationship between corneal densitometry and CT.

RESULTS

In the study, we prospectively assessed 32 eyes of 17 patients (8 men and 9 women) with newly diagnosed POAG. Mean age was 47.5±7.3 years (37-61 years) in the patient group. There were 14 subjects (6 men and 8 women) and mean age was 45.2±6.3 years (35-60 years) in the control group. There was no significant difference in age and gender between the patient and control groups (p=0.281 and p=0.675, respectively).

Table 1 presents CT, IOP and VA values at baseline and on days 3, 7 and 30 after treatment. The CT was significantly higher in central zones of 2, 6 and 10 mm on day 3 after treatment when compared to baseline (p<0.001 for all). However, there was no significant difference in CT between baseline and days 7 or 30 after treatment (p>0.05 for all). The IOP was decreased significantly on day 3 after treatment when compared to baseline (24.5±3.9 ve 17.0±3.5, p<0.001). There were no significant differences between IOP values on days 3, 7 and 30 after treatment (p=0.467, variance analysis). Again, there was no significant difference between VA values obtained at baseline and after treatment (p>0.05 for all).

Table 1 summarizes corneal densitometry values at baseline and on days 3, 7 and 30 after treatment in the patient group. On day 3 after treatment, central densitometry values in all 3 zones (0-2, 6-10 and 6-10 mm) were significantly higher when compared to baseline (p=0.012, p=0.001 and p=0.001, respectively). However, there were no significant differences in densitometry values between baseline and on days 7 or 30 after treatment (p> 0.05 for all).

Table 2 summarizes comparison of corneal thickness and densitometry values between patient and control groups. On day 3, the corneal thicknesses in zones of 2, 6 and 10 mm were significantly higher in the patient group when compared to healthy controls (p<0.001 for all). On day 3 after treatment, central densitometry values in all 3 zones (0-2, 6-10 and 6-10 mm) were significantly higher in the patient group when compared to healthy controls (p=0.008, p=0.002 and p=0.001, respectively).

On day 3, no significant correlation was found between corneal densitometry value of central layer in 0-2 mm zone and CT in corneal circle of 2 mm (r=0.223, p=0.219) or between corneal densitometry value of central layer in 2-6 mm zone and CT in corneal circle of 6 mm (r=0.332, p=0.348).

No ocular (pain, blurred vision, photophobia, pricking etc.) or systemic adverse effect was observed throughout treatment.

DISCUSSION

Ocular hypotensive effects on ciliary body as well as corneal and retinal effect have been reported for brimonidine.[3, 4] There is limited number of studies about central corneal thickness increase with brimonidine in the literature while there is no in vivo study evaluating corneal changes in patients treated with brimonidine. In this study, it was aimed to determine the corneal layers of which corneal changes originate in patients treated with topical brimonidine 0.01% preparation and the effects of such changes on cornea densitometry. This is the first study which objectively assessed short-term effect of topical brimonidine preparation on corneal transparency using Pentacam. Our results indicated that, in eyes treated with brimonidine, increased corneal densitometry values in central layer of all three corneal zone, which were increased on day 3 and returned to baseline values on day 7 after treatment.

Table 1: *VA, IOP, CT and corneal densitometry changes at baseline and after treatment in the patient group.*

	Baseline	Day 3	p^*	Day 7	p^{**}	Day 30	p^{***}
VA	0.087±0.1	0.088±0.1	*0.325*	0.088±0.1	*0.328*	0.088±0.1	*0.326*
IOP	24.5±3.9	17.0±3.5	***<0.001***	17.3±3.6	***<0.001***	16.9±3.2	***<0.001***
CT							
0	552.3±35.3	566.5±35.4	***<0.001***	553.9±34.2	*0.138*	551.1±32.6	*0.846*
2	582.1±38.6	598.3±40.7	***<0.001***	583.5±33.1	*0.235*	581.1±34.5	*0.624*
6	620.6±52.5	635.8±54.1	***<0.001***	619.4±56.2	*0.185*	620.2±54.2	*0.578*
10	774.3±98.1	793.5±101.2	***<0.001***	775.2±97.5	*0.321*	776.6±100.1	*0.265*
CD							
Anterior 120 µm							
0-2	21.84±2.81	22.06± 2.69	*0.214*	21.64± 2.19	*0.623*	22.09± 2.18	*0.352*
2-6	23.09±2.46	23.59± 2.33	*0.471*	23.52± 2.18	*0.512*	23.14± 2.36	*0.625*
6-10	31.25±5.52	32.21± 6.23	*0.070*	31.68± 5.21	*0.418*	32.08± 5.23	*0.116*
Central							
0-2	13.56±3.40	15.71± 5.17	***0.012***	14.12± 3.24	*0.108*	13.46± 4.21	*0.312*
2-6	15.18±2.66	18.43± 4.47	***0.001***	16.25± 3.02	*0.084*	14.52± 4.65	*0.213*
6-10	17.78±2.26	21.02± 4.27	***0.001***	17.34± 3.58	*0.256*	18.12± 3.05	*0.114*
Posterior 60 µm							
0-2	10.03±1.97	10.21± 2.21	*0.206*	11.14± 3.18	*0.108*	10.14± 2.11	*0.258*
2-6	11.31±2.42	12.84± 3.33	*0.121*	12.08± 2.87	*0.211*	11.18± 2.25	*0.184*
6-10	14.25±2.65	16.28± 3.02	***0.010***	15.12± 3.14	*0.102*	14.36± 2.24	*0.156*

VA: corrected visual acuity (logMAR), **CT:** corneal thickness (µm), **IOP:** intraocular pressure (mmHg), **CD:** corneal densitometry: Gray scale unit, *: Comparison between day 3 and baseline (dependent t test), **: Comparison between day 7 and baseline (dependent t test), ***: Comparison between day 30 and baseline (dependent t test)

Table 2: *Comparison of changes in corneal thickness and densitometry values between patient and control groups.*

	Baseline			Day 3			Day 30		
	Patient	**Control**	**p^***	**Patient**	**Control**	**p^{**}**	**Patient**	**Control**	**p^{***}**
CT									
0	552.3±35.3	550.6±29.7	*0.163*	566.5±35.4	550.4±28.3	***<0.001***	551.1±32.6	550.2±29.6	*0.128*
2	582.1±38.6	579.8±34.1	0.07	598.3±40.7	579.1±33.2	***<0.001***	581.1±34.5	578.9±30.7	*0.109*
6	620.6±52.5	623.2±49.6	0.216	635.8±54.1	623.5±47.3	***<0.001***	620.2±54.2	623.4±45.3	*0.251*
10	774.3±98.1	780.2±99.7	0.101	793.5±101.2	780.6±92.7	***<0.001***	776.6±100.1	781.3±92.9	*0.186*
CD									
Anterior 120 µm									
0-2	21.84±2.81	23.41±2.23	*0.106*	22.06± 2.69	23.38±2.35	*0.142*	22.09± 2.18	23.40±2.58	*0.184*
2-6	23.09±2.46	22.68±2.56	*0.235*	23.59± 2.33	21.78±2.73	*0.117*	23.14± 2.36	22.18±2.61	*0.287*
6-10	31.25±5.52	32.28±5.34	*0.385*	32.21± 6.23	31.29±5.37	*0.219*	32.08± 5.23	33.42±5.21	*0.254*
Central									
0-2	13.56±3.40	12.86±3.06	*0.162*	15.71± 5.17	12.52±3.15	***0.008***	13.46± 4.21	13.10±2.98	*0.172*
2-6	15.18±2.66	15.25±3.47	*0.219*	18.43± 4.47	15.34±3.28	***0.002***	14.52± 4.65	14.87±3.59	*0.114*
6-10	17.78±2.26	17.36±2.58	*0.431*	21.02± 4.27	17.43±2.28	***0.001***	18.12± 3.05	17.49±2.76	*0.267*
Posterior 60 µm									
0-2	10.03±1.97	10.17±1.63	*0.625*	10.21± 2.21	10.21±1.56	*0.512*	10.14± 2.11	11.12±1.74	*0.492*
2-6	11.31±2.42	12.21±2.74	*0.147*	12.84± 3.33	12.34±2.54	*0.181*	11.18± 2.25	12.75±2.56	*0.196*
6-10	14.25±2.65	15.16± 3.18	*0.103*	16.28± 3.02	15.32± 3.43	*0.09*	14.36± 2.24	14.85± 3.41	*0.113*

CT: corneal thickness, CD: corneal densitometry, *: Comparison of baseline and healthy controls (independent t test)
: Comparison of day 3 and healthy controls (independent t test), *: Comparison of day 30 and healthy controls (independent t test)

Table 3: *Comparison of corneal thickness and densitometry values between patient and control groups.*

	Patient group			Control group			
	Baseline	**Day 3**	**Day 30**	**Control**	***p****	***p****	***p*****
Corneal thickness							
Central	552.3±35.3	566.5±35.4	551.1±32.6	550.6±29.7	*0.163*	***<0.001***	*0.128*
2 mm	582.1±38.6	598.3±40.7	581.1±34.5	579.8±34.1	0.07	***<0.001***	*0.109*
6 mm	620.6±52.5	635.8±54.1	620.2±54.2	623.2±49.6	0.216	***<0.001***	*0.251*
10 mm	774.3±98.1	793.5±101.2	776.6±100.1	780.2±99.7	0.101	***<0.001***	*0.186*
Corneal densitometry							
Anterior 120 µm							
0-2 mm	21.84±2.81	22.06± 2.69	22.09± 2.18	23.41±2.23	*0.106*	*0.142*	*0.184*
2-6 mm	23.09±2.46	23.59± 2.33	23.14± 2.36	22.68±2.56	*0.235*	*0.117*	*0.287*
6-10 mm	31.25±5.52	32.21± 6.23	32.08± 5.23	32.28±5.34	*0.385*	*0.219*	*0.254*
Central							
0-2 mm	13.56±3.40	15.71± 5.17	13.46± 4.21	12.86±3.06	*0.162*	***0.008***	*0.172*
2-6 mm	15.18±2.66	18.43± 4.47	14.52± 4.65	15.25±3.47	*0.219*	***0.002***	*0.114*
6-10 mm	17.78±2.26	21.02± 4.27	18.12± 3.05	17.36±2.58	*0.431*	***0.001***	*0.267*
Posterior 60 µm							
0-2 mm	10.03±1.97	10.21± 2.21	10.14± 2.11	10.17±1.63	*0.625*	*0.512*	*0.492*
2-6 mm	11.31±2.42	12.84± 3.33	11.18± 2.25	12.21±2.74	*0.147*	*0.181*	*0.196*
6-10 mm	14.25±2.65	16.28± 3.02	14.36± 2.24	15.16± 3.18	*0.103*	*0.09*	*0.113*

*: Comparison between baseline and healthy control (Independent t test), **: Comparison between day 3 and healthy control (Independent t test), ***: Comparison between day 30 and healthy control (Independent t test)

It is well-known that topical therapies used in glaucoma lead local adverse effects due to its active substance or excipients. Thus, the prevalence of ocular surface disease is extremely higher in patients with glaucoma as a result of several factors such as need for multi-drug therapies. In animal studies, it was shown that protective agents such as benzalkonium (BAK) have toxic effect on ocular surface; impair tear film stability; influence on ocular surface function; and harm cornea, conjunctiva and trabecular cells.[9-11] Alphagan-P eye drop contains benzalkonium chloride as well as boric acid, calcium chloride dihydrate, magnesium chloride hexahdyrate, potassium chloride, sodium borate decahydrate, sodium carboxymethyl cellulose, sodium chloride, hydrochloride acid and/or sodium hydroxide and purified water. Allergic conjunctivitis, blepharitis and conjunctival hyperemia are among drug-related adverse events previously reported after brimonidine use.[12, 13] However, corneal disorders related to brimonidine use are rather rare and underlying mechanisms haven't been elucidated.[14-16] Maruyama et al. reported sterile corneal infiltration and corneal opacity associated to chronic brimonidine use.[16] Authors suggested that it may have been due to allergic reaction against brimonidine-associated antibodies since they successfully treated corneal infiltrations with steroids. Kasuya et al. reported that bilateral brimonidine-related corneal stromal opacity may occur due to allergic reaction, interaction between brimonidine and other anti-glaucomatous agents, effects of protective agents such benzalkonium or sodium chloride and racial variations.[17]

Brimonidine is rapidly absorbed by cornea following topical administration and acts on alpha-2-adrenoreceptors in corneal epithelium and endothelium.[18, 19] Grueb et al. evaluated thicknesses of corneal layers in patients treated with topical brimonidine 0.1% using anterior segment OCT and reported that there was increased stromal and epithelial thickness in central zone, which increased on day 2 and returned normal on day 5 after topical administration.[4] Cankurtaran et al. reported increased central CT in healthy eyes given single dose of brimonidine 0.15%.[20] It is though that brimonidine causes increase in corneal thickness due to adverse effect on endothelial pump mechanism secondary to activation of endothelial alpha-2-adrenoreceptors.[4] In our study, central CT was increased on day 3 and returned to normal on day 7 after topical administration in agreement with literature (Table 1). In addition, it was shown that there was increased thickness in zones of 2, 6 and 10 mm on day 3 after treatment This indicates that topical

brimonidine effects endothelial and epithelial functions in all corneal zones. It is thought that fluid passage to stromal bed is blocked due to rapid receptor desensitization and/or activation of alpha-2 antagonist receptors after topical administration; followed by re-establishment of corneal compensation. Thus, corneal thickness values returned to baseline values on day 7 after treatment (Table 1).

Corneal transparency depends on regulation of fluid balance within corneal stromal layer. Fluid influx to cornea is balanced by active ion and fluid transport capacity at endothelium and epithelium. Corneal epithelium and endothelium ensure corneal transparency by acting as barrier to fluid passage and active ion domains.[21, 22] In corneal epithelium and endothelium, chlorine ion transport is regulated via interaction between positive effects induced by stimulation of beta and alpha-1 receptors and negative effects induced by stimulation of alpha-2 receptors. Thus, corneal beta receptor stimulation will provide corneal fluid loss and increase corneal transparency by inducing active ion transportation. On contrary, corneal alpha-2 adrenergic stimulation will inhibit the system and decrease corneal transparency by reducing ion transportation and increasing fluid retention in stromal bed. In our study, increased corneal densitometry values were obtained in central corneal layer in all three zones on day 3 after topical brimonidine 0.1% administration (Table 1). No significant difference was detected in anterior and posterior corneal layers when compared to baseline. These findings indicate that alpha-2 adrenergic receptors stimulated by topical brimonidine increase fluid retention central corneal layer farthermost to endothelial and epithelial layers by reducing ion transportation.

In our study, corneal densitometry and CT values returned to baseline values on day 7 after topical brimonidine. This finding suggest that corneal compensation is re-established due to rapid receptor desensitization and activation of other receptors counteracting to alpha-2 receptors. In an animal study, Polat et al. showed that topical beta adrenoreceptor and alpha adrenoreceptor agonists did not lead significant changes in basal lamina, stroma and Descemet membrane in rabbit cornea at long-term. However, authors found mild ultra-structural changes such as vacuolization due to enlarged focal lytic areas in cytoplasm and endoplasmic reticulum cisternae in corneal endothelium.[23] In our study, no significant difference was observed in corneal densitometry values on day when compared to baseline and healthy controls.

No significant difference was found in VA values obtained at baseline and on days 3, 7, 30. This finding indicates that the decreased corneal transparency on day 3 isn't clinically relevant regarding visual acuity. In addition, it was thought that corneal transparency might be affected by IOP values decreased on day 3 but showed no significant difference on day 3, 7 and 30. However, corneal densitometry values were increased on day 3 and returned to baseline values on day 7 after treatment. Thus, it was concluded that there was no correlation between IOP and change in corneal densitometry.

Although this is the first study evaluating effects of brimonidine on corneal transparency, the study has some limitation including small sample size and shorter follow-up duration. Another limitation is that evaluations regarding effects of changes in corneal densitometry on contrast sensitivity is lacking.

In conclusion, topical brimonidine 0.1% administration might lead to temporary increase in corneal thickness and corneal densitometry values in all zones at short-term. It is thought that the temporary effect of brimonidine on cornea may be due to fluid retention, particularly in central corneal layer. Although the effect that decreases on corneal transparency seems not to be significant, larger studies with longer follow-up are needed to investigate clinical relevance of these alterations.

REFERENCES

1. Fudemberg, S.J., Batiste, C., Katz, L.J. Efficacy, safety, and current applications of brimonidine. Expert Opin. Drug Saf. 2008;7:975-9.
2. Reitsamer H.A., Posey M., Kiel J.W. Effects of a topical alpha2 adrenergic agonist on ciliary blood flow and aqueous production in rabbits. Exp. Eye Res. 2006;82:405-15.
3. Kalapesi F.B., Coroneo M.T., Hill M.A. Human ganglion cells express the alpha-2 adrenergic receptor: relevance to neuroprotection. Br. J. Ophthalmol. 2005;89:758-63.
4. Matthias G., Joerg M., Jens Martin R., et al. Effect of brimonidine on corneal thickness. 2011;27:503-9
5. Ozyol P, Ozyol E. Assessment of corneal backward light scattering in diabetic patients. Eye Contact Lens. 2018;44:92-6.
6. Ni Dhubhghaill S, Rozema JJ, Jongenelen S, et al. Normative values for corneal densitometry analysis by Scheimpflug optical assessment. Invest Ophthalmol Vis Sci 2014;55:162-8.
7. Lopes B, Ramos I, Ambrósio R Jr. Corneal densitometry in keratoconus. Cornea. 2014;33:1282-6.
8. Oliveira CM, Ribeiro C, Franco S. Corneal imaging with slit-scanning and Scheimpflug imaging techniques. Clin Exp Optom. 2011;94:33-42.
9. Becquet F, Goldschild M, Moldovan MS, et al. Histopathological effects of topical ophthalmic preservatives on rat corneoconjunctival surface. Curr Eye Res. 1998;17:419-25.

10. Noecker RJ, Herrygers LA, Anwaruddin R. Corneal and conjunctival changes caused by commonly used glaucoma medications. Cornea. 2004;23:490- 6.

11. Hamard P, Debbasch C, Blondin C, et al. Human trabecular cells and apoptosis: in vitro evaluation of the effect of betaxolol with or without preservative. J Fr Ophthalmol. 2002;25:777-84.

12. Osborne SA, Montgomery DM, Morris D, et al. Alphagan allergy may increase the propensity for multiple eye-drop allergy. Eye (Lond). 2005;19:129-37.

13. Manni G, Centofanti M, Sacchetti M, et al. Demographic and clinical factors associated with development of brimonidine tartrate 0.2%- induced ocular allergy. J Glaucoma. 2004;13:163-7.

14. Manabe Y, Sawada A, Mochizuki K. Corneal sterile infiltration induced by topical use of ocular hypotensive agent. European Journal of Ophthalmology. 2020;30:23-5.

15. Tsujinaka A, Takai Y, Inoue Y, et al. A case of bilateral deep stromal corneal opacity and vascularization after use of multiple antiglaucoma medications including brimonidine tartrate ophthalmic solution. Acta Ophthalmologica, 2019;97:948-9.

16. Maruyama Y, Ikeda Y, Yokoi N et al. Severe corneal disorders developed after brimonidine tartrate ophthalmic solution use. Cornea 2017;36:1567-9.

17. Kasuya Y, Sano I, Makino S et al. Corneal Opacity Induced by Antiglaucoma Agents Other Than Brimonidine Tartrate. Case Rep Ophthalmol Med. 2020 May 26;2020:4803651. doi: 10.1155/2020/4803651.

18. Grueb M, Bartz-Schmidt K U, Rohrbach J M. Adrenergic regulation of cAMP/protein kinase A pathway in corneal epithelium and endothelium. Ophthal. Res. 2008;40:322-8.

19. Grueb M, Wallenfels-Thilo B, Denk O. Monoamine receptors in human corneal epithelium and endothelium. Acta Ophthalmol. Scand. 2006;84:110-5.

20. Cankurtaran V, Tekin K. Effects of a Single Dose of Topical Brimonidine 0.15% on Anterior Segment Morphology, Pupil Characteristics, and Choroidal Thickness in Healthy Subjects. Eye Contact Lens. 2020 May 14. doi: 10.1097/ICL.0000000000000708. Online ahead of print.

21. Bonanno, J.A. Identity and regulation of ion transport mechanisms in the corneal endothelium. Prog. Retin. Eye. Res. 2003;22:69-94.

22. Edelhauser, H.F. The balance between corneal transparency and edema. Invest. Ophthalmol. Vis. Sci. 2006;47:1755-67.

23. Polat S, Ozdemir N, Soylu M, et al. Ultrastructural effects of topical beta-adrenergic antagonists and an alpha-adrenergic agonist on the rabbit cornea. Ophthalmologica 1999;213:183-8.