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Original Article

Evaluation of Corneal Endothelial Cell Morphology Using Specular Microscopy and Corneal Topographic Parameters in Children with Hyperopic Anisometropic Amblyopia

Ulviye Kıvrak, Sibel Öskan Yalçın, Ayşin Tuba Kaplan, Elif Sarı, Burak Tanyıldız

Department of Ophthalmology, Health Sciences University Kartal Dr. Lutfi Kirdar City Hospital, İstanbul, Türkiye

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Correspondence: Ulviye Kıvrak, Kartal Dr. Lutfi Kirdar City Hospital, Istanbul, Türkiye
E-mail: dr.ulviyekivrak@hotmail.com

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ABSTRACT

Objective: To evaluate anterior segment parameters in pediatric patients with hyperopic anisometropic amblyopia (HAA).

Methods: The study included 35 amblyopic and 35 healthy eyes of 35 HAA patients examined in the Pediatric Ophthalmology Department between January and April 2021 and 40 eyes of 40 healthy emmetropic children. The axial length (AL) was measured using a Lenstar LS-900 biometer, and the anterior segment and corneal endothelial layer parameters were evaluated using Scheimpflug imaging and non-contact specular microscopy, respectively.

Results: The mean age of the children was 8.5±3.1 years in the HAA group and 8.9±2.2 years in the healthy control group (p=0.252). A statistically significant difference was found in the three groups regarding best-corrected visual acuity and AL (p<0.001 for all). Of the anterior segment parameters, a statistically significant difference was determined between the groups in terms of anterior corneal curvature, corneal volume, anterior chamber depth, total high-order aberrations, and spherical aberration (p=0.019, p=0.034, p=0.015, p=0.037, and p<0.001, respectively). No statistically significant difference was determined between the groups concerning the endothelial parameters (p>0.05 for all).

Conclusion: The results of this study demonstrated that there could be statistically significant differences in anterior segment parameters between amblyopic eyes, fellow eyes, and emmetropic healthy eyes. A thorough assessment of anterior segment parameters in patients can significantly inform both diagnostic and therapeutic approaches.

INTRODUCTION

Amblyopia is a decrease in unilateral or bilateral best-corrected visual acuity (BCVA) that occurs due to vision loss or abnormal binocular interaction during the critical period of vision development.[1] It is among the most frequent reasons for loss of visual acuity in pediatric patients.[1] Amblyopia may develop associated with strabismus, anisometropia, bilateral high refractive error, or visual deprivation. In cases where the etiology cannot be identified, it is called idiopathic amblyopia.[2]

Anisometropia develops due to a significant refractive error between the two eyes, with the amblyogenic effect being more significant in hyperopic anisometropia than in myopic and astigmatic anisometropia.[3] Spheric and astigmatic changes occur due to some anatomic differences in the axial length and corneal and lenticular curvature between the two eyes. Treatment includes optic correction, eye patching, and medications such as atropine and levodopa.[4] However, even with the correct diagnosis, treatment success has been reported in only 60-75% of cases.[5]

Several studies addressed the relationship of amblyopia with the axial length (AL), topographic parameters of the cornea, and corneal high-order aberrations (HOA), with the latter having an adverse effect on treatment results.[6-8] However, no study has evaluated the relationship between amblyopia and changes in endothelial parameters as measured by non-contact specular microscopy.

Specular microscopy, a noninvasive imaging technique, is used to assess the morphology of the corneal endothelium, the innermost layer of the cornea that is responsible for corneal transparency.[9] Changes in the corneal endothelium may occur due to ocular diseases as well as systemic diseases.[9] These changes in corneal endothelial cells affect not only the corneal parameters but also visual quality and acuity.[10] Corneal endothelial changes may not always be apparent during routine ophthalmic examination. Fung et al.[11] reported no lesions in 13% of the patients with posterior polymorphous corneal dystrophy and lower endothelial cell density (ECD) in these children compared to healthy controls. Specular microscopy, in this respect, may play an essential role in the assessment of corneal endothelial changes.

This study aimed to evaluate the anterior segment parameters, especially the corneal endothelial parameters, of the amblyopic eyes of pediatric patients with hyperopic anisometropic amblyopia (HAA) compared to fellow healthy eyes and emmetropic healthy eyes.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Pediatric Ophthalmology between January and April 2021 and included patients with HAA aged 5-15 years. The study's approval was obtained from the Ethics Committee of Kartal Dr. Lütfi Kırdar City Hospital (Protocol No: 2021/514/205/2), and our study was performed according to the principles of the Declaration of Helsinki. It was not possible to obtain written consent for study participation because it was a retrospective design.

The diagnosis of HAA was based on a discrepancy of >1.00 diopter (D) (spherical equivalent) between the two eyes and a disparity of >two rows in BCVA based on Snellen visual acuity charts, with a BCVA of ≥0.63 in the fellow eye in patients younger than six years, and ≥0.8 in patients aged ≥six years. The spherical equivalents were calculated following cycloplegic refraction (spherical power + ½ cylinder power). The refraction measurements were made 45 minutes after administering 1% cyclopentolate hydrochloride twice at 5-minute intervals. The control groups consisted of one randomly selected emmetropic eye of age- and sex-matched children with normal ocular findings on routine ophthalmologic examinations. Emmetropia was defined as an uncorrected visual acuity (UCVA) of >0.8 on a Snellen chart.

Exclusion criteria included a history of any ocular disease (strabismus, nystagmus, uveitis, retinal and corneal diseases, cataract, optic disc anomalies, and diseases, or glaucoma), any history of ocular or head trauma, intraocular surgery, the presence of astigmatism >1D, the presence of any neurologic disease or developmental retardation, or inability to cooperate during eye examinations.

All participants underwent a detailed eye examination, including UCVA and BCVA on the Snellen chart (the Snellen equivalent was converted to logMAR), an alternating cover test, intraocular pressure (IOP) measured using an air-puff tonometer, a slit-lamp biomicroscopic examination, and dilated fundus evaluations. Laterality (eye with anisometric hyperopic amblyopia) was identified in patients with HAA.

The AL, defined as the distance between the retinal pigment epithelium and the corneal vertex, was measured three times by the same experienced technician using a Lenstar LS-900 biometer (LS 900 Haagstreit AG; Koeniz, Switzerland) and was averaged. Before each measurement, the participant was instructed to blink the eye to obtain an integrated tear film layer.

The same skilled technician measured the anterior segment parameters, including anterior and posterior corneal curvatures (ACC and PCC, respectively), central corneal thickness (CCT), corneal volume (CV), and anterior chamber depth (ACD) and volume (ACV) using Scheimpflug imaging technology (Sirius Topography, CSO, Florence, Italy). The root mean squares of the total HOAs within 6 mm of the central cornea were evaluated while the pupil was at the physiologic size in the mesopic condition.

The same technician used a non-contact specular microscope (Tomey EM-4000; Tomey Corp.) to measure the corneal endothelial cells. The center approach was used to repeat each measurement at least three times, with a minimum of 110 cells in each measurement. The values recorded for all patients were the endothelial cell density (ECD), average cell area (ACA), minimum cell area (CAmin), maximum cell area (CAmax), standard deviation of cell area (SD), coefficient of variation in cell area (CV), and hexagonal cell ratio (HEX).

Statistical Analysis

The study's data were examined using the statistical program Jamovi 2.2.5. Depending on the distribution, continuous data were presented as standard deviation or median (interquartile range) values. The independent samples t-test was used to compare groups based on age, and the Chi-square test was used to compare genders. The homogeneity and normalcy of the distribution were evaluated using the Shapiro-Wilk and Kolmogorov-Smirnov tests. In the comparisons between groups, continuous data with a normal distribution were subjected to a one-way analysis of variance (ANOVA), while non-normal data were subjected to the Kruskal-Wallis test. After a significant result from a one-way ANOVA, a multiple comparison test called the post-hoc Games-Howell test was employed. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

This study included 70 eyes of 35 patients with HAA and 40 eyes of 40 emmetropic healthy control subjects. The HAA group consisted of 19 males and 16 females, and the healthy control group included 18 males and 22 females. The mean ages of the two groups were 8.51±3.08 and 8.92±2.16 years, respectively. Regarding gender and age, there was no significant difference between the HAA and healthy control groups ($p=0.252$ and $p=0.951$, respectively). Most (68%) of the eyes with HAA were on the left side. The control group eyes were 55% right-side and 45% left-side (Table 1).

Table 1. Clinical and demographic characteristics of participants.

	All participants (n=75)	Participants With Amblyopia (n=35)	Participants With no Amblyopia (n=40)	P
Age, (years)				
mean±sd	8.73±2.62	8.51±3.08	8.92±2.16	0.252
M (min-max)	(4-16)	(4-16)	(4-14)	
Gender, n (%)				
Female	34 (45.3%)	19 (54.3 %)	22 (55%)	0.951
Male	41 (54.7%)	16 (45.7%)	18 (45%)	
Laterality of amblyopia (Right/Left)	11/64	11/24	22/18	

No pathology was detected in the slit-lamp biomicroscopic and dilated fundus examinations of any study participants. In the study, there were three groups of eyes included: amblyopic eyes of patients with HAA, non-amblyopic fellow eyes of patients with HAA, and emmetropic eyes of healthy controls. There was a statistically significant difference in the refractive errors following cycloplegic refraction, BCVA, and AL when comparing amblyopic eyes with both fellow eyes and healthy eyes (p<0.001 for all).

Among the anterior segment measurements, statistically significant differences were found between the groups for ACC, CV, and ACD (p=0.019, p=0.034, and p=0.015, respectively). The ACC and CV values showed a statistically significant difference (p=0.035, p=0.046) between the amblyopic eyes and the control group, and the ACD values showed a statistically significant difference between the amblyopic eyes and both the non-amblyopic fellow eyes as well as the control group eyes (p=0.045, p=0.045).

The CCT values were found to be higher in the amblyopic eyes than in the control group, but the difference was not

Table 2. Comparison of refractive and corneal parameters of participants.

	Participants With Amblyopia (n=35)		Participants With no Amblyopia (n=40)	P	Post-hoc
	Amblyopic Eye	Non Amblyopic Fellow Eye	Non Amblyopic Control Eye		
Refraction, D, SE, mean±sd	+5.31±1.92	+3.35±2.23	+0.05±1.17	<0.001	1&3 p<0.001 2&3 p<0.001 1&3 p<0.001
BCVA (logMAR)	0.30±0.32	0.00	0.00	<0.01	1&3 p<0.001 1&2 p<0.001
IOP, mmHg	14.7±2.17	14.8±2.89	15.7±1.95	0.071	
CCT, (µm), mean±sd	581±43.3	585±48.4	563±37.8	0.059	
AL (mm)	22.2±1.39	23.2±0.94	23.3±0.94	<0.001	1&3 p<0.001 1&2 p=0.004
ACC, D	59.5±3.81	59.7±3.53	57.7±3.55	0.019	1&3 p=0.035
PCC, D	-5.53±2.82	-5.97±0.34	-5.81±1.92	0.053	
CV, mm^3	43.6±1.29	42.8±1.51	42.7±1.59	0.034	1&3 p=0.046
ACD, mm	3.50±0.34	3.50±0.34	3.68±0.25	0.015	1&3 p=0.045 2&3 p=0.045
Total HOA	0.15±0.16	0.09±0.07	0.07±0.02	0.037	1&3 p=0.024
Coma	0.08±014	0.03±0.03	0.04±0.06	0.295	
Spherical Aberation	0.09±0.34	0.05±0.03	0.01±0.008	<0.001	1&3 p<0.001
Trefoil	0.06±0.06	0.04±0.04	0.05±0.03	0.155	
Pupil Diameter, mm	3.53±0.72	3.62±0.71	3.45±0.51	0.066	

D: Diopters; SE: Spherical equivalent; BCVA: Best corrected visual acuity; IOP: Intraocular pressure; CCT: Central corneal thickness; AL: Axial lenght, ACD: Anterior corneal depth; CV: Corneal volüme; HOA: High order aberration; ACC: Anterior corneal curvature; PCC: Posterior corneal curvature; 1: ambliyopic eye; 2: Non ambliyopik fellow eye; 3: Non ambliyopic control eye.

Table 3. Comparison of specular microscopy parameters of participants

	Participants With Amblyopia (n=35)		Participants With no Amblyopia (n=40)	P
	Amblyopic Eye	Non Amblyopic Fellow Eye	Non Amblyopic Control Eye	
ECD (cells/mm)	3126±332.0	3122±326.0	3039±240.0	0.312
ACA	324.0±36.9	324.0±36.7	331.0±26.1	0.498
SD	111.0±30.2	111.0±25.5	114.0±23.7	0.822
CV	33.9±6.42	34.1±5.51	34.3±5.51	0.970
HEX	55.4±9.64	55.4±10.1	56.4±11.0	0.904

ECD: Endothelial cell density; ACA: Average cell area; SD: Standard deviation of cell area; CV: Coefficient of variation in cell area; HEX: Hexagonal cell ratio.

statistically significant (p=0.059). Of the HOAs, the total HOA and spherical aberration values were statistically significantly different between the three groups (p=0.037 and p<0.001, respectively). Particularly between the amblyopic eyes and the healthy control group, there was a difference (p=0.024, p<0.001) (Table 2).

The endothelial parameters measured using specular microscopy are shown in Table 3. There was no statistically significant difference among the groups (p>0.05 for all).

DISCUSSION

The results of this study demonstrated differences in the anterior segment parameters of the amblyopic eyes of patients with HAA compared with the fellow non-amblyopic eyes and the healthy control group. To the best of our knowledge, this is the first study to evaluate the endothelial parameters of patients with HAA in addition to AL and corneal topographic parameters. HAA is seen at an equal frequency in males and females.[7,12] Although some studies reported that it was more common in males, the frequency was similar in both genders in the current study.[7] Some studies reported that amblyopia was seen more often in the left eye in HAA, and even rates two-fold higher compared with the right eye have been reported.[8] This has been associated with the fact that ocular dominance is seen more often in the right eye in the general population. It is thought that to prevent diplopia arising from image disparity, ocular dominance leads to interocular suppression, albeit at a low level.[13] Romboust et al.[14] showed that the dominant eye in a healthy individual activated a broader visual cortex, which was thought to be linked to a more effective emmetropisation process in the eye with ocular dominance.

The optical and anatomical developmental stages and mechanisms of the eye are not entirely known, but there are various opinions on emmetropisation.[15] The hyperopic eye in newborn infants progresses towards emmetropisation over time. This change is rapid in the first three years and tends to decrease over time, with AL reaching the adult level at the age of 15 years.[16] During this period, the refractive power of the cornea and lens decreases, and a decrease in hyperopia is observed. Consequently, while the sagittal axis of the eye lengthens in the emmetropisation process, the ACD and vitreous chamber depth deepen, and flattening of the cornea and lens occurs due to equatorial stretching.[16] Emmetropisation cannot be completed when there is a delay in development in this sensitive period, which triggers the development of amblyopia. Previous studies have shown that AL is significantly shorter in eyes with HAA.[15,17] Cass and Tromans reported a disproportionate increase in lens and corneal thickness and shortening of the AL, attributing this to the developmental delay in emmetropisation.[17] Some studies suggested this condition was due to insufficient visual quality in the anisometropic amblyopic eye, affecting AL through a negative feedback mechanism.[18] Therefore, it can be postulated that a bidirectional interaction exists between ocular structures and the emmetropisation process. In this study, AL was observed to be statistically significantly shorter in amblyopic eyes, and the ACD in HAA eyes and the fellow eyes was found to be significantly shallower than in the healthy emmetropic eyes of the control group, which we attributed to the inability to complete the emmetropisation process correctly in the amblyopic eye.

Wang et al.[19] found no statistically significant difference among the anterior segment parameters of amblyopic eyes and fellow eyes. They stated that this situation may develop because the fellow eye may not be normal in patients with HAA. Unlike this study, Yuksel et al.[6] included a third group of healthy emmetropic children and observed no statistically significant difference among the groups in their study. In contrast to these studies, Cankurtaran et al.[7] reported thicker CCT in anisometropic hyperopic eyes than in fellow eyes. They attributed this to the abnormal emmetropisation process and the absence of flattening and thinning of the cornea due to equatorial stretching that would be expected to occur during ocular sagittal expansion. In this study, both the CCT of the amblyopic eye and the fellow eye were thicker than the control group, but they were not found to be statistically higher. These results also revealed that the evaluation of the fellow eye as normal may not be completely accurate and that the delay in the emmetropisation process may have an effect on CCT differences. At the same time, the ACC and CV parameters were significantly higher in the HAA eyes than in the control group. CV shows a tendency to decrease with aging and has been reported to be associated with parameters such as ACD, CCT, anterior corneal asphericity, and PCC, especially in inverse proportion to AL and the white-to-white distance.[20] The significantly shorter AL and proportionately thicker CV in HAA eyes in the current study were thought to be related to each other.

HOAs are corneal aberrations that affect visual quality, and their increase can lead to symptoms such as halo, glare, and distortion.[10] The correction of HOAs increases visual acuity and contrast sensitivity.[8,21] Whereas low-order aberrations show a 90% effect on retinal image quality, there is a 10% effect on HOAs such as coma, spherical aberrations, and trefoil.[22] Prakash et al.[23] compared amblyopic eyes with healthy eyes and reported a significant difference in HOA patterns. Some studies have attributed this to the amblyogenic effect of the change in HOAs.[22,24] In contrast, it has also been claimed that there is no relationship between amblyopia and HOAs.[25,26] However, one of these studies, Levy et al.'s [25] study, included adult patients with amblyopia, and it is known that HOAs may change with aging. Brunette et al.[27] reported that ocular aberrations reduced slowly in early adulthood, reaching the lowest level in the fourth decade of life, and then at advanced ages, showed an increase associated with changes in the lens.

In a study by Dominguez-Vicent et al.,[26] no relationship was reported between idiopathic amblyopia and HOAs, but remarkably few patients were included in that study. In contrast, it has been claimed that HOAs are more effective than the amblyogenic effect on the relationship between amblyopia and the treatment success of amblyopia in some studies.[21,22] Kwan et al.[28] showed that coma and spherical aberration were particularly common in healthy eyes. Zhao et al.[22] reported that coma affected amblyopia. Previous studies have shown a significant relationship between coma and astigmatism.[21,27] Choi et al.[21] reported a statistically significant difference in spherical aberration in eyes with hyperopic amblyopia. The current study found a statistically significant difference in total HOA and spherical aberration in eyes with HAA. This difference was especially seen between the amblyopic and healthy control group eyes. It is known that corneal aberrations are twice as common in infants than in adults, and together with aging, progress towards emmetropisation is similar to low-order aberrations.[29] In this study, it was thought that the differences in total HOA and spherical aberration were related to abnormalities in the emmetropisation process in amblyopic eyes, and there was no statistically significant difference in coma because the patients in the current study were patients with <1D astigmatism.

In humans, corneal endothelial cells are stopped in the G1 phase of the cell cycle.[30] Due to this limited proliferative capacity, visual function and quality may be significantly compromised following disease or injury related to corneal endothelial loss. In addition to the reduction in the quantity of corneal endothelial cells, changes in endothelial cell morphology also affect visual quality. Patients with Fuchs' endothelial corneal dystrophy (FECD) have been reported to experience visual impairment even in the absence of corneal stromal or epithelial edema. Corneal endothelial morphological changes can adversely affect visual quality by inducing light scattering. Furthermore, in individuals with FECD, there is a proportional relationship between the corneal endothelial morphological changes and measures such as corrected distance visual acuity, letter contrast sensitivity, and visual quality related to light scattering.[29]

Therefore, changes in the corneal endothelial layer that occur during the sensitive period when emmetropization develops in childhood may cause amblyopia. It has been reported that astigmatic anisometropic amblyopia developed due to endothelial changes in patients with posterior polymorphous corneal dystrophy, which causes changes in the corneal endothelial layer.[12] There was no discernible difference observed in the current study between the groups when endothelial morphology was examined using non-contact specular microscopy. This study includes only the hyperopic anisometropic patient group; however, examining the endothelial parameters in all amblyopia cases (hyperopic, myopic, and astigmatic anisometropic amblyopia cases) may provide insights into idiopathic amblyopia cases whose causes remain unexplained.

The limited sample size, retrospective design, absence of long-term follow-up, and lack of comparisons between amblyopia treatment and outcomes constitute several of the study's limitations. Additionally, patients with amblyopia due to refractive errors other than hypermetropia were not included.

Conclusion

In conclusion, amblyopia is one of the most common causes of vision loss; however, it is preventable and treatable in childhood. Understanding the pathophysiology of HAA, which is seen more often in children, is extremely important for preventing and treating the disease. The results of this study demonstrated that there could be statistically significant differences between amblyopic eyes, fellow eyes, and healthy eyes in terms of anterior segment parameters. As a result, a comprehensive evaluation of the anterior segment parameters of patients could provide valuable guidance for both diagnosis and treatment.

Ethics Committee Approval

The study was approved by the Kartal Dr. Lütfi Kırdar City Hospital Ethics Committee (Date: 12.07.2021, Decision No: 2021/514/205/2).

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: U.K., B.T., A.T.K.; Design: U.K., S.Ö.Y., B.T., A.T.K.; Supervision: U.K., B.T., A.T.K.; Materials: U.K., S.Ö.Y., A.T.K., E.S.; Data collection &/or processing: U.K., E.S., S.Ö.Y., A.T.K., B.T.; Analysis and/or interpretation: U.K., B.T., A.T.K.; Literature search: U.K., E.S., S.Ö.Y., B.T., A.T.K.; Writing: U.K., A.T.K.; Critical review: U.K., S.Ö.Y., A.T.K., E.S., B.T.

Conflict of Interest

None declared.

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Hipermetrop Anizometropik Ambliyopili Çocuklarda Kornea Endotel Hücre Morfolojisinin ve Korneal Topografik Parametrelerin Değerlendirilmesi

Amaç: Hipermetrop anizometropik ambliyopili (HAA) pediatrik hastalarda ön segment parametreleri değerlendirildi.

Gereç ve Yöntem: Çalışmaya Ocak-Nisan 2023 tarihleri arasında pediatrik göz hastalıkları kliniği'nde muayene edilen 35 HAA hastasının 35 ambliyopik ve 35 sağlıklı gözü ile 40 sağlıklı emetrop çocuğun 40 gözü dahil edildi. Aksiyal uzunluk (AL), Lenstar LS-900 biyometresi kullanılarak ölçüldü. Korneal anterior segment ve endotel tabakası parametreleri sırasıyla Scheimpflug görüntüleme ve temassız speküler mikroskopi kullanılarak değerlendirildi.

Bulgular: Çocukların ortalama yaşı HAA grubunda 8.5±3.1 yıl, sağlıklı kontrol grubunda ise 8.9±2.2 yıl idi (p=0.252). En iyi düzeltilmiş görme keskinliği ve AL açısından üç grup arasında istatistiksel olarak anlamlı fark bulundu (p<0.001). Anterior segment parametrelerinde gruplar arasında ön kornea eğriliği, kornea hacmi, ön kamara derinliği, toplam yüksek dereceli aberasyonlar ve sferik aberasyonlar açısından istatistiksel olarak anlamlı farklılık olduğu saptandı (sırasıyla, p=0.019, p=0.034, p=0.015, p=0.037 ve p<0.001). Endotel parametreleri açısından gruplar arasında istatistiksel olarak anlamlı bir fark saptanmadı (tümü için p>0.05).

Sonuç: Bu çalışma, ambliyopik hastalarda ambliyopik gözler ve diğer sağlıklı gözler ile emetropik sağlıklı gözler arasında ön segment parametrelerinde istatistiksel olarak anlamlı farklılıklar olabileceğini göstermiştir. Hastalarda ön segment parametrelerinin ayrıntılı değerlendirilmesi hem tanı hem de tedavi yaklaşımlarını önemli ölçüde etkileyebilir.

Anahtar Sözcükler: Aksiyal uzunluk; hipermetrop anizometropik ambliyopi; kornea topografisi; speküler mikroskopi; yüksek dereceli sapma.