Quantitative analysis of retinal microcirculation in children with hyperopic anisometropic amblyopia: an optical coherence tomography angiography study

Sibel Doğuizi, MD, Meltem Yılmazoğlu, MD, Hasan Kızıltoprak, MD, Mehmet Ali Şekeroğlu, MD, and Pelin Yılmazbaş, MD

PURPOSE To evaluate optical coherence tomographic angiography (OCTA) findings on retinal microcirculation in hyperopic anisometropic amblyopia compared with fellow eyes and nonamblyopic control eyes.

METHODS A total of 40 pediatric patients with hyperopic anisometropic amblyopia and 57 control subjects were recruited, and 137 eyes (40 amblyopic, 40 fellow, and 57 control eyes) were evaluated. Data on best-corrected visual acuity (logMAR), axial length (mm), refractive error, and OCTA findings (foveal avascular zone parameters, macular vascular density in superficial and deep retinal capillary plexus, central macular thickness) were recorded in amblyopic, fellow, and control eyes.

RESULTS Compared with fellow and control eyes, amblyopic eyes were associated with significantly lower foveal vessel density values within 300 μm around the foveal avascular zone ($P < 0.01$) and lower vascular density in certain areas of superficial and deep retinal capillary plexus in axial length- and refraction-adjusted analysis ($P < 0.05$ for all), along with significantly higher full thickness of the central macula ($P = 0.04$). In amblyopic eyes, best-corrected visual acuity values were negatively correlated with foveal density ($r = -0.57$; $P = 0.02$) and deep capillary retinal plexus vascular density in foveal ($r = -0.51$; $P = 0.03$) parafovea temporal ($r = -0.52$; $P = 0.03$), and parafovea superior ($r = -0.51$; $P = 0.04$) areas.

CONCLUSIONS Our findings indicate a possible association between retinal microcirculation and amblyopia. (J AAPOS 2019;23:201.e1-5)

Hyperopic anisometropia is one of the leading causes of unilateral amblyopia.[1-3] Although amblyopia is associated with a structurally normal eye, several studies suggest that subclinical abnormalities of the eye and visual pathways may exist in amblyopic eyes.[4,5] Recent studies have aimed to analyze macular thickness, optic nerve morphology, and choroidal thickness in the amblyopic eye.[6,7]

Evaluation of the vascular system of the retina is also of interest in terms of potential clinical and prognostic value of vascular parameters in patients with macular diseases.[8]

Author affiliation: Ulucanlar Eye Training and Research Hospital, Department of Ophthalmology, Ankara, Turkey

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Correspondence: Sibel Doğuizi, MD, Department of Ophthalmology, Ulucanlar Eye Training and Research Hospital, Altindag, 06230, Ankara, Turkey (email: eryigits@yahoo.com).

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Optical coherence tomographic angiography (OCTA) is a relatively new and noninvasive imaging modality that permits visualization of the microvasculature of the retina throughout its depth, allowing for the identification of the superficial retinal capillary plexus and the deep retinal capillary plexus.[8] Although macular findings by OCTA in patients with amblyopia have been extensively studied, most of these studies have focused on abnormalities related to foveal thickness and differences in the retinal layers such as the retinal nerve fiber layer; these studies have obtained inconsistent and controversial findings.[5,7-9]

Macular OCTA findings consistent with changes in microvasculature of the retina in amblyopic eyes have been reported in several small-scale studies.[8,10,11] Apart from a few studies on anatomic characterization of peripapillary nerve fiber layer, retina, and choroid structures in hyperopic anisometropic amblyopic eyes,[12,13] no studies to date, to our knowledge, have addressed the macular OCTA findings specific to hyperopic anisometropic amblyopia. This study was designed to investigate potential changes in retinal microcirculation in children with hyperopic anisometropic amblyopia in terms of foveal avascular zone parameters and macular vessel density findings on OCTA in amblyopic eyes compared with fellow eyes and age-matched control eyes.

Subjects and Methods

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Numune Eye Training and Research Hospital Ethics Committee. Written informed consent was obtained from the parents or legal guardian of each patient following a detailed explanation of the study objectives and protocol.

A total of 40 pediatric patients with hyperopic anisometropic amblyopia (mean age, 13.0 ± 3.8 years; 24 males) and 57 control subjects (mean age, 14.2 ± 2.8 years; 34 males) were recruited, and 137 eyes (40 amblyopic and 40 fellow eyes of patients; 57 control eyes) were evaluated in this prospective cross-sectional tertiary care center study. For the amblyopic and control eyes, the mean best-corrected visual acuity was 0.45 ± 0.24 logMAR and −0.01 ± 0.03 logMAR, the mean spherical equivalent refraction was 5.31 ± 1.39 and 1.09 ± 0.72, and the mean axial length was 21.1 ± 0.68 mm and 22.4 ± 0.57 mm, respectively (Table 1). The amblyopic eyes were treated with refractive correction alone for 16 weeks and, if needed, with refractive correction and patching afterward.

Inclusion criteria were presence of unilateral amblyopia due to hyperopic anisometropia for the children in the amblyopia group. The control group was composed of age- and sex-matched children with normal ocular findings admitted to our ophthalmology outpatient clinic for a routine ocular examination. Presence of strabismus, deprivation amblyopia, nystagmus, history of previous ocular surgery, intraocular inflammation, corneal disease, retinal disease or optic nerve disease, ocular media opacities, including cataract or other diseases of the visual pathway, glaucoma, neurological disease, and insufficient cooperation were exclusion criteria. All refractive errors were converted to spherical equivalent values with consideration of anisometropia for an interocular difference of at least 1.50 D in the spherical equivalence. Unilateral amblyopia was defined as best-corrected visual acuity of 20/30 or worse in one eye without organic cause for the decreased vision and at least a 2-line difference in interocular visual acuity between the amblyopic eye and the fellow eye. Only hyperopic anisometropic amblyopic eyes with visual acuity of 20/30 or worse in one eye and a fellow normal eye with visual acuity of 20/20 were included. All control subjects also underwent a complete eye examination and were required to have best-corrected visual acuity of 20/20. Subjects with a history of prematurity or systemic conditions that could alter the microvasculature (including diabetes, hypertension, and renal disease) were also excluded.

Study Parameters

Data on best-corrected visual acuity (logMAR), axial length (mm), refractive error, and OCTA findings (foveal avascular zone parameters [area, perimeter, acircularity index, and foveal density], macular vascular density in superficial and deep retinal capillary plexus, and central macular thickness) were recorded and compared between amblyopic, fellow, and control eyes. Foveal density refers to the vessel density in an area of 300 μm around the foveal avascular zone. The correlation between OCTA and other ocular examination findings was analyzed in amblyopic eyes.

A comprehensive eye examination was performed, including best-corrected visual acuity with Snellen charts (converted to logMAR for analysis), slit-lamp examination, intraocular pressure measurement, cover and cover-uncover testing, ocular motility testing, and fundus examination. Two drops of cyclopentolate hydrochloride 1% were administered 5 minutes apart, and autorefraction was performed using automatic refractor-keratometer device (Canon RF-K2, Tokyo, Japan) approximately 45 minutes after the last drop. Axial length was measured using a LenStar LS-900 device (LS 900 Haag-Streit AG; Koeniz, Switzerland) by the same investigator (MY).

Imaging

All OCTA scans were performed and assessed for vessel density and foveal avascular zone parameters by the same experienced retina specialist (SD) using the AngioVue device (version 2017.1.0.151 of the RTVue XR Avanti, Opto-Vue Inc, Fremont, CA). Each scan was repeated until the signal strength was ≥8 (on a 10-point scale); results with poor image quality, low signal strength index (<8), presence of blink artifacts, and poor fixation leading to motion or doubling artifacts on OCTA were excluded. The OCTA system used in the study is based on split-spectrum amplitude decorrelation angiography algorithm and operates at 70,000 A-scans per second to acquire OCTA volumes consisting of 400 × 400 A-scans. All scans of each eye had 6 mm × 6 mm scanning area centered on fovea.

Each scan was automatically segmented by the software to visualize the superficial capillary plexus and deep capillary plexus of the retina (Figure 1). The foveal zone was defined in the area of the small circle (1 mm in diameter), the parafoveal zone was defined in the area of the middle circle (3 mm in diameter), and the perifoveal zone was defined in the area of the outer circle (6 mm in diameter). Additionally, the zones were automatically divided into four equal quadrants (temporal, nasal, inferior, and superior) and two equal hemispheres (superior and inferior) (Figure 1). Data on area, perimeter, and acircularity index (defined as the ratio of the perimeter of the foveal avascular zone to the perimeter of a circle with equal area) of the foveal avascular zone and foveal density were also automatically obtained (Figure 2).

Central macular thickness was calculated by an automated algorithm that measures retinal thickness from the inner limiting membrane to the retinal pigment epithelium, inner retinal thickness from inner limiting membrane to the inner plexiform layer, and outer retinal thickness from inner plexiform layer to the retinal pigment epithelium (Figure 2).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp, Armonk, NY). The consistency between the variants and normal distribution was investigated by means of visual (histogram and probability

Table 1. Comparison of best-corrected visual acuity, axial length, and refractive error parameters in amblyopic eyes, fellow eyes, and control eyes

	Amblyopic eyes (n = 40), mean ± SD (range)	Fellow eyes (n = 40), mean ± SD (range)	Control eyes (n = 57), mean ± SD (range)	*P* value[a]
BCVA, logMAR	0.45 ± 0.24 (0.20 to 1.00)[b]	−0.01 ± 0.02 (−0.12 to 0.00)	−0.01 ± 0.03 (−0.12 to 0.00)	<0.001
Axial length, mm	21.1 ± 0.68 (20.0 to 22.3)[b]	22.3 ± 0.48 (21.0 to 23.0)	22.4 ± 0.57 (21.0 to 23.7)	<0.001
Refractive error				
Sphere	4.96 ± 1.43 (2.50 to 8.75)[b]	1.41 ± 0.90 (0.25 to 3.75)	1.02 ± 0.57 (0 to 2.25)	<0.001
Cylinder	0.51 ± 0.41 (0 to 1.25)	0.46 ± 0.40 (0 to 1.25)	0.46 ± 0.35 (0 to 1.25)	0.23
SE	5.31 ± 1.39 (2.75 to 8.25)[b]	1.53 ± 1.09 (0 to 4.25)	1.09 ± 0.72 (0 to 2.25)	<0.001

BCVA, best-corrected visual acuity; *SD*, standard deviation; *SE*, spherical equivalent.
[a]Kruskal-Wallis test.
[b]Statistically significant difference compared with fellow and control eyes in post hoc binary comparison (the Bonferroni correction, with a *P* value of <0.017 considered statistically significant, was applied for multiple comparisons).

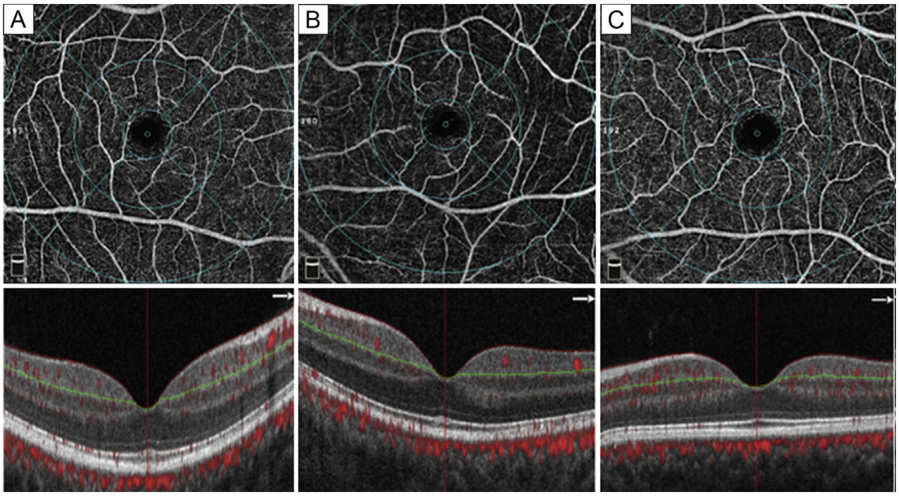

FIG 1. Optical coherence tomographic angiography (OCTA) 6 × 6 mm macular scans. Vessel density in the superficial retinal capillary plexus (SCP) of the normal fellow eye of a patient with amblyopia (A), the amblyopic eye of the same patient (B), and a control eye of a healthy subject (C).

graphics) and analytical methods (Kolmogorov-Smirnov Test/Shapiro-Wilk Test). Categorical variants were assessed using the Pearson χ^2 test. The Mann-Whitney *U* test and the Kruskal-Wallis test with post hoc binary comparisons were used for analysis of non-normally distributed variables; whereas analysis of variance with Tukey HSD and Tamhane *T2* test for post hoc binary comparisons was used for normally distributed variables. One-way covariance analysis was used for axial length– and refraction-adjusted analysis. Correlation between OCTA and other ocular examination findings was analyzed using Spearman correlation analysis. Data were expressed as mean with standard deviation, and minimum-maximum values and percentage (%) where appropriate. A *P* value of <0.05 was considered statistically significant. For the adjustment of *P* value for multiple comparisons, Bonferroni correction was used (*P* = 0.05/number of comparisons).

Results

No significant difference was noted between patients with amblyopic eye and healthy controls in terms of age ($P = 0.15$), sex ($P = 0.13$), and laterality of the eye (left in 26 [65%] patients vs 27 [47%] controls; $P = 0.09$). Best-corrected visual acuity and spherical equivalent refractive error values were significantly higher and axial length was significantly lower in amblyopic eyes compared with fellow and control eyes ($P < 0.001$ for each). See Table 1.

OCTA Findings

Considering foveal avascular zone parameters, no significant difference was noted between amblyopic, fellow, and control eyes in terms of area, perimeter, and acircularity index, whereas mean foveal density values were significantly lower in amblyopic eyes as compared with fellow and control eyes: 54.8 ± 3.2 (range, 45.9-60.2) vs 56.7 ± 2.57 (range, 48.8-59.9) and 57.1 ± 3.5 (range, 50.5-64.3), respectively ($P < 0.01$). These findings persisted after adjustment for axial length– and refraction-adjusted analysis, with significantly lower foveal density values in the amblyopic eyes ($P < 0.01$) and no significant difference between groups in terms of area, perimeter, and acircularity index. Considering macular vascular

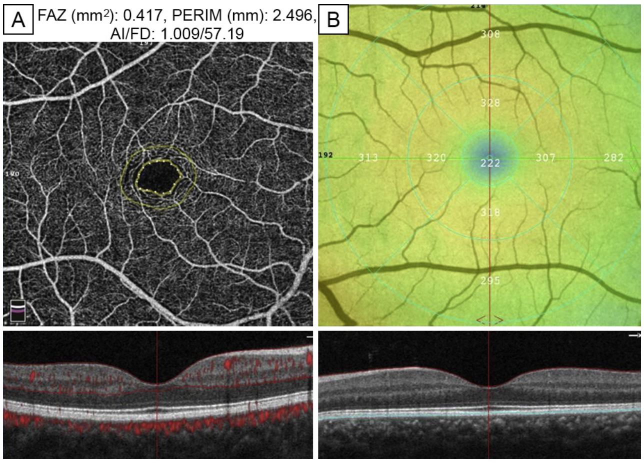

FIG 2. Foveal avascular zone (FAZ) and retinal thickness measurements of a healthy eye obtained using built-in software of OCTA. A, FAZ area (mm^2) in whole retina, FAZ perimeter (mm), acircularity index of the FAZ, and foveal density (AI/FD). B, Retinal thickness measurements of automatically divided zones.

density, vascular density in the superficial retinal capillary plexus including whole retina, superior-hemi, foveal, parafoveal (overall, superior-hemi, superior, temporal, and inferior), and perifoveal (overall, superior-hemi, and superior) areas were significantly lower in amblyopic eyes compared with fellow and control eyes ($P < 0.05$ for all). These findings persisted following axial length- and refraction-adjusted analysis at all areas ($P < 0.05$ for all), except for the parafovea-inferior region ($P = 0.15$). The macular vascular density in the deep retinal capillary plexus including whole retina, inferior-hemi, foveal, parafoveal (overall, inferior-hemi, superior, temporal, and inferior) and perifoveal (overall, inferior-hemi, temporal, and inferior) areas were significantly lower in amblyopic eyes as compared with fellow and control eyes ($P < 0.05$, for all). These findings persisted following axial length– and refraction-adjusted analysis at all areas ($P < 0.05$ for all) except for the perifovea-inferior region ($P = 0.06$).

Considering macular thickness, mean full thickness of the central macula was significantly higher in amblyopic eyes than in fellow and control eyes: 249.6 ± 12.8 (range, 207.0-265.0) vs 244.1 ± 14.9 (range, 205.0-295.0) and 243.1 ± 12.6 (range, 205.0-262.0), respectively; $P = 0.03$. No significant difference was noted between groups in terms of inner and outer macular thickness. These findings persisted after axial length– and refraction-adjusted analysis with significantly higher macular full thickness ($P = 0.04$) in amblyopic eyes, whereas there was a similar inner and outer macular thickness between groups.

No statistically significant difference was noted between fellow and control eyes in terms of OCTA findings before and after axial length– and refraction-adjusted analysis ($P > 0.05$ for all).

OCTA and Other Findings in Amblyopic Eyes

In amblyopic eyes, no significant correlation of axial length and refraction was noted with OCTA findings, whereas best-corrected visual acuity values were negatively correlated with foveal density ($r = -057$; $P = 0.02$) and deep capillary retinal plexus vascular density in foveal ($r = -0.51$; $P = 0.03$), parafovea temporal ($r = -0.52$; $P = 0.03$), and parafovea superior ($r = -0.51$; $P = 0.04$) areas.

Discussion

Our findings revealed an association between amblyopic eyes and lower foveal density values, lower vascular density in certain regions of superficial and deep capillary plexus, and higher central macula thickness. Best-corrected visual acuity was negatively correlated with foveal density values as well as with deep retinal vascular density in certain areas.

There are a limited number of studies on retinal vascular structure in amblyopic eyes.[8,10,11] Guo and colleagues[10] reported no significant difference was reported between normal and amblyopic eyes in terms of vascular density at the superficial capillary plexus or deep capillary plexus in 44 eyes of 22 patients with unilateral amblyopia due to strabismus or anisometropia. In contrast, Lonngi and colleagues[8] compared 13 unilateral amblyopic eyes due to strabismus or anisometropia with 50 normal eyes and found significantly lower vascular density at the superficial capillary plexus and deep capillary plexus of amblyopic eyes compared with controls. Yilmaz and colleagues[11] reported significantly lower vascular density in the superficial and deep retinal capillary plexus in 15 amblyopic eyes (strabismic or anisometropic) compared with contralateral and control eyes.

Our findings revealed significantly lower vascular density in the foveal, parafoveal, and perifoveal areas of the superficial capillary plexus and deep capillary plexus in hyperopic anisometropic amblyopic eyes compared with fellow and control eyes before and after axial length– and refraction-adjusted analysis. The concordance of our findings in hyperopic anisometropic amblyopia with other studies on amblyopia caused by strabismus or anisometropia indicates a change in retinal microvasculature, regardless of the etiology of amblyopia.[8,11]

It is well known that retinal and vascular parameter measurements are associated with axial length and refraction values. Li and colleagues[14] reported a negative correlation of retinal vascular density with axial length and myopic refractive values. Given that amblyopic eyes were hypermetropic in our study, the refraction was higher and axial length was lower in amblyopic eyes than in fellow and control eyes. Considering the potential influence of these differences on retinal vascular parameters, we adjusted for refraction and axial length. Significant association between vascular density and amblyopia persisted after adjustment in certain regions.

Previous studies on the retinal changes in patients with amblyopia revealed association of amblyopia with a wide range of retinal parameters, including central retinal thickness, peripapillary nerve fiber thickness, ganglion layer thickness, outer nuclear layer thickness, outer segment length, and subfoveal choroid thicknesses.[12,15-20] The variety of the results may be due to the heterogeneity of the patient populations in terms of age, race, and amblyopia type as well as a lack of analysis adjusted for refraction and axial length in amblyopic eyes.

Foveal avascular zone parameters other than the foveal avascular zone area, such as acircularity index, perimeter, and foveal density, were investigated for the first time in amblyopic eyes in our study. Our findings support lack of a significant difference in foveal avascular zone area in amblyopic eyes reported in past studies,[8,11] while also indicating no significant difference between amblyopic eyes, fellow eyes, and control eyes in terms of acircularity index and perimeter values and significantly lower foveal density values in amblyopic eyes. Hence, our findings suggest that amblyopia is associated with vascular intensity in the foveal region.

Several studies show an association between a reduction in vascular density in the foveal region, particularly at the deep capillary plexus, and visual acuity.[21-23] Usui and colleagues[24] demonstrated that a healthy vascular structure in the deep capillary plexus and foveal region was closely related with visual acuity.

Our study shows a negative correlation of best-corrected visual acuity with foveal density values and vascular density at foveal, parafoveal temporal, and parafoveal superior regions of the deep capillary plexus. Our findings also suggest that decrease in visual acuity is related to the decrease in foveal density and vascular density of the deep capillary plexus in amblyopic eyes.

Literature Search

PubMed was searched, without language/date restriction, using the following terms: *amblyopia*, *optical coherence tomographic angiography*, *hyperopic anisometropic amblyopia*, and *retinal microvasculature*.

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