Association of Optical Coherence Tomography Angiography Metrics With Detection of Impaired Macular Microvasculature and Decreased Vision in Amblyopic Eyes

The Hong Kong Children Eye Study

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**IMPORTANCE** Microvascular abnormalities in amblyopia are becoming evident with high-resolution imaging, such as optical coherence tomography angiography (OCT-A); however, to our knowledge, the clinical significance and use of these findings are unknown.

**OBJECTIVE** To assess changes in quantitative OCT-A metrics in amblyopic eyes and explore their association with visual acuity in children.

**DESIGN, SETTING, AND PARTICIPANTS** This population-based nested case-control study included children aged 6 to 8 years who were consecutively recruited between January 2016 and July 2017 from the population-based Hong Kong Children Eye Study (HKCES) at the Chinese University of Hong Kong Eye Centre. All participants underwent OCT-A with a swept-source OCT and detailed ophthalmic investigations. Macular microvasculature of the superficial capillary plexus was quantified by a customized automated image analysis program. A multivariable linear regression was conducted to evaluate the differences in OCT-A metrics between amblyopic and nonamblyopic eyes after adjustment for all known confounders. Data analysis was conducted from September to November 2018.

**MAIN OUTCOMES AND MEASURES** Differences in OCT-A metric (foveal avascular zone [FAZ]) area, FAZ circularity, vessel density, vessel diameter index, and fractal dimension between amblyopic and nonamblyopic eyes.

**RESULTS** There were 30 participants with amblyopia (mean [SD] age, 7.57 [1.2] years; 16 girls [53.3%]) and 1045 controls (mean [SD] age, 7.65 [1.0] years; 580 girls [55.5%]) in this cohort. Compared with control eyes, amblyopic eyes had decreased FAZ circularity (−0.058; 95% CI, −0.096 to −0.021; \( P = .002 \)), decreased fractal dimension (−0.014; 95% CI, −0.024 to −0.003; \( P = .01 \)), and increased vessel diameter index (0.002; 95% CI, 0.002 to 0.003; \( P < .001 \)). A difference was not identified between FAZ area and vessel density. LogMAR visual acuity was associated with FAZ circularity (\( \beta = −0.133; \ P < .001 \)) and vessel diameter index (\( \beta = 0.097; \ P = .001 \)) but not with vessel density nor FAZ area.

**CONCLUSIONS AND RELEVANCE** The results of this population-based study in children support the presence of macular microvascular abnormalities in amblyopic eyes. Such changes as measured by OCT-A metrics are associated with visual acuity, inferring retinal involvement in the development of amblyopia and suggesting a potential role of quantitative OCT-A metrics in the diagnosis and recognition of amblyopia.
Amblyopia is one of the most commonly encountered ophthalmic conditions in children, with the population-based prevalence ranging from 0.5% to 3.5%. Strabismus, anisometropia, and visual deprivation are common risk factors of amblyopia thought to result in functional and morphological effects on the visual cortex and lateral geniculate nucleus. Recent studies have started to look into the involvement of the retina in the development of amblyopia with evaluations of retinal nerve fiber layer, macular and choroidal thickness, and ganglion cell complex thickness with intriguing yet inconsistent results. Up to now, the exact nature and structural-functional correlation of retinal microvasculature involvement in amblyopia has not been well defined.

With the increased availability of optical coherence tomography angiography (OCT-A), detailed microvascular networks can now be quantified quickly and noninvasively. Several studies using OCT-A in children have reported abnormal microvasculature in amblyopic eyes compared with normal controls. However, because of heterogenous study designs and the presence of several confounders of OCT-A, the results have been inconsistent. Furthermore, these abnormal OCT-A parameters have not been demonstrated to be associated with visual acuity, raising questions regarding the clinical significance of these findings in amblyopia.

In this study, we used a fully automated custom MATLAB (MathWorks) program to measure several unitless OCT-A metrics from OCT angiograms. These metrics quantify the extent of microvascular damages sensitively with good repeatability and reproducibility and have been widely used in our previous studies. To eliminate bias, all known ocular and systemic confounders were adjusted. We also explored whether macular microvasculature measured by OCT-A metrics is associated with visual acuity.

**Methods**

**Study Population**

Data for this analysis were derived from the Hong Kong Children Eye Study (HKCES), a population-based cohort study of eye conditions in children from first grade to third grade (aged about 6-8 years) from primary schools in Hong Kong. In brief, the HKCES was designed to determine the prevalence of various eye disorders in children, including refractive errors, strabismus, amblyopia, and allergic eye diseases, and identify their environmental and genetic determinants. Sample selection was based on a stratified and clustered randomized sampling frame. In Hong Kong, all primary schools (n = 571) registered in the Education Bureau were stratified into 7 cluster regions according to population densities. This division into 7 cluster regions is determined by the Hong Kong government according to an even distribution of population density in each cluster region. In HKCES, the schools in each cluster region were randomly assigned an invitation priority according to the ranking numbers generated by computer. Invitations to participate in the cohort were sent according to the ranking numbers until the required sample was achieved in each cluster region.

**Key Points**

**Question** Are macular microvascular abnormalities in children with amblyopia still present after adjusting for all known confounders and do they correlate with visual acuity?

**Findings** In this population-based nested case-control study of 30 children with amblyopia and 1045 controls, amblyopic eyes had abnormal macular microvascular represented by decreased foveal avascular zone circularity, decreased fractal dimension, and increased vessel diameter index. LogMAR visual acuity correlated with foveal avascular zone circularity and vessel diameter index.

**Meaning** Quantitative optical coherence tomography angiography metrics detect impaired microvasculature in amblyopic eyes that correlates with visual acuity, suggesting a potential role in amblyopia assessment.

This study included a subpopulation of HKCES, and participants were consecutively recruited from January 2016 to July 2017 to undergo OCT-A imaging. All children were ethnic Chinese. Exclusion criteria included the presence of deprivation amblyopia, nystagmus, retinal diseases, intraocular inflammation, and media opacity, such as corneal disease or cataract. Patients with a history of prematurity, neurologic disease, or systemic conditions that could alter the microvasculature were excluded. In addition, patients with head, neck, or other injuries preventing proper positioning or with an inability to maintain retinal fixation were excluded.

The study adhered to the Declaration of Helsinki and the study protocol was approved by the ethics committee board of the Prince of Wales Hospital, the Chinese University of Hong Kong. All parents signed an informed consent document on participation in the study. Participants were not offered payment or incentives to participate in the study.

**Definitions of Amblyopia**

Amblyopia was defined as a best-corrected visual acuity (BCVA) between 20/40 and 20/200 OD/OS or OU without an identifiable organic cause for the decreased vision. Only strabismic or anisometropic amblyopia were included in this study. For patients with bilateral amblyopia, the eye with poorer vision was selected for OCT-A imaging.

Children with a BCVA of 20/20 or greater were included in the control group after completing a full ophthalmic examination with no evidence of any ocular abnormality. Only the right eye was chosen for analysis. If the right eye OCT-A image was excluded from analysis during quality control or missing, measurement would be performed on the left eye. The fellow eye of patients with unilateral amblyopia was not included in the control group even if the BCVA of fellow eye was 20/20 or greater.

**Ophthalmic Examinations**

All children underwent a full ophthalmic examination by an ophthalmic specialist. Visual acuity (VA) was measured with (aided VA) or without eyeglasses (unaided VA) using a logMAR chart (Nidek). Refractive status was measured before and after cycloplegia using an autorefractor (Nidek ARK-510A). Two cycles of cyclopentolate, 1% (Alcon), and tropicamide, 1%...
Quantification of Retinal Microvasculature

A customized automated MATLAB program (2017 version; MathWorks) was used for all image analyses. The details of the quantification of retinal capillary network from OCT-A and its reliability assessment have been reported before. The area of the FAZ (mm²) was calculated by counting the total numbers of pixels within the region in scale. The FAZ circularity index was calculated as the ratio of the measured area of FAZ to the area of the projected circle (same perimeter as the identified FAZ) with a range of 0 to 1. A ratio closer to 0 indicated an irregular shape of FAZ, whereas closer to 1 indicated a circular shape. Total vessel density (VD) was calculated as the percentage of area not defined as nonperfusion regions over the total area excluding the central Early Treatment Diabetic Retinopathy Study 1-mm circle. The binarized image was also skeletonized and the fractal dimension (FD) was then calculated by using the box-counting method. Vessel diameter index (VDI) was calculated as the area occupied by blood vessels from the binarized image over the total length of blood vessels from the skeletonized image.

Statistical Analysis

Statistical analysis was performed using SPSS, version 24 (IBM). An independent-sample t test was used to compare baseline demographics and evaluate differences in OCT-A metrics between amblyopic and nonamblyopic eyes. The OCT-A metrics (FAZ area, FAZ circularity, VD, FD, and VDI) were log-transformed because of their skewed distribution. A 2-sided P value of .05 was used to test for statistical significance. Linear regression analyses were performed to determine BCVA associations with log-transformed OCT-A metrics (independent variables) after adjustment for confounders, including age, sex, body mass index (calculated as weight in kilograms divided by height in meters squared), spherical equivalent, AL, OCT-A quality score, and central subfield thickness. We compared standardized regression coefficients (sβ) with higher sβ values, indicating stronger associations with OCT-A metrics.

Results

A total of 1218 participants were recruited for this study. Of these, 143 (11.7%) were excluded because of low-quality scores (30 [21.0%]), motion artifacts (45 [31.5%]), blurry images or tilted images (60 [42.0%]), signal loss (5 [3.5%]), and poor centration (3 [2.1%]), leaving a total of 1075 eyes from 1075 participants for the final analysis. The mean (SD) age of the study participants was 7.64 (0.98; range, 5.52-8.77) years. There were 530 boys (49.3%) and 545 girls (50.7%). Among them, 30 (2.8%) had amblyopia and 1045 (97.2%) were controls. There were 3 cases of bilateral amblyopia, and the worse eye was chosen for analysis. Comparative analyses of the baseline demographic characteristics of the participants in this report (OCT-A cases) with those in the whole HKCES did not show a difference (eTable in the Supplement).

Table 1 shows the demographic characteristics of the amblyopic cases and controls. The baseline characteristics, including age, sex, AL, body mass index, central subfield thickness, and quality score of OCT-A imaging, were not different between groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Control eyes (n = 1045)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>7.57 (1.20)</td>
<td>7.65 (0.97)</td>
</tr>
<tr>
<td>Sex, male/female, No.</td>
<td>14/16</td>
<td>465/580</td>
</tr>
<tr>
<td>BCVA, logMAR (Snellen equivalent)</td>
<td>0.31 (20/40) (0.13)</td>
<td>0.02 (20/20) (0.06)</td>
</tr>
<tr>
<td>Spherical equivalent, diopter</td>
<td>-0.09 (4.34)</td>
<td>-0.65 (1.26)</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>23.10 (1.38)</td>
<td>23.18 (0.90)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>16.34 (2.77)</td>
<td>16.32 (3.52)</td>
</tr>
<tr>
<td>OCT-A quality score</td>
<td>66.63 (6.37)</td>
<td>66.85 (3.77)</td>
</tr>
<tr>
<td>Central subfield thickness, μm</td>
<td>257.98 (21.76)</td>
<td>264.07 (18.68)</td>
</tr>
</tbody>
</table>

Abbreviations: BCVA, best-corrected visual acuity; OCT-A, optical coherence tomography angiography.

* Independent-samples t test did not show difference between groups in terms of baseline characteristics. As expected, there was a difference between the spherical equivalent of amblyopic eyes and control eyes, which was adjusted as a covariate in the following analysis.

* Calculated as weight in kilograms divided by height in meters squared.
As expected, there was a difference between the spherical equivalent of amblyopic eyes and control eyes. Histogram plots of OCT-A metrics showed skewed distribution. Therefore, log-transformed OCT-A metrics were used in a linear regression analysis. Table 2 shows the comparison between the 2 groups in terms of FAZ, FAZ circularity, fractal dimension, VDI, and VD after adjustment for age, sex, AL, quality score of OCT-A, and central subfield thickness. The mean (SD) FAZ circularity was lower in the amblyopic group compared with controls (0.549 [0.145] vs 0.611 [0.103]; $P = .002$), indicating an irregular shape of the FAZ. Furthermore, the amblyopic eyes had a lower mean (SD) FD (1.721 [0.015] vs 1.734 [0.028]; $P = .01$) and larger mean (SD) VDI (0.021 [0.005] vs 0.018 [0.003]; $P < .001$) when compared with the control eyes. However, we did not find a difference between amblyopic eyes and control eyes in mean (SD) FAZ area (0.290 [0.118] mm² vs 0.323 [0.137] mm²; $P = .24$) nor mean (SD) VD (97.030 [2.020] vs 97.200 [1.660]; $P = .61$).

**Table 2. Multivariable Analysis Comparing Amblyopic Eyes vs Control Eyes in Terms of FAZ, FAZ Circularity, Fractal Dimension, Vessel Diameter Index, and Vessel Density**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) Amblyopic eyes (n = 30)</th>
<th>Mean (SD) Control eyes (n = 1045)</th>
<th>Difference between amblyopia and control eyes (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAZ, mm²</td>
<td>0.290 (0.118)</td>
<td>0.323 (0.137)</td>
<td>−0.027 (−0.071 to 0.018)</td>
<td>.24</td>
</tr>
<tr>
<td>FAZ circularity</td>
<td>0.549 (0.145)</td>
<td>0.611 (0.103)</td>
<td>−0.058 (−0.096 to −0.021)</td>
<td>.002</td>
</tr>
<tr>
<td>Fractal dimension</td>
<td>1.721 (0.015)</td>
<td>1.734 (0.028)</td>
<td>−0.014 (−0.024 to −0.003)</td>
<td>.01</td>
</tr>
<tr>
<td>Vessel diameter index</td>
<td>0.021 (0.005)</td>
<td>0.018 (0.003)</td>
<td>0.002 (0.002 to 0.003)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vessel density, %</td>
<td>97.030 (2.020)</td>
<td>97.200 (1.660)</td>
<td>−0.170 (−0.701 to 0.400)</td>
<td>.61</td>
</tr>
</tbody>
</table>

Abbreviation, FAZ, foveal avascular zone.

*Adjusted for optical coherence tomography angiography quality score, age, sex, spherical equivalent, central subfield thickness, and body mass index (calculated as weight in kilograms divided by height in meters squared).
described previously (Table 3). Poorer visual acuity was correlated with reduced FAZ circularity ($\beta$, $-0.133; P < .001$) and increased vessel diameter index ($\beta$, $0.097; P = .001$). Best-corrected visual acuity was not associated with FAZ area ($\beta$, $-0.045; P = .09$), FD ($\beta$, $-0.029; P = .35$), and VD ($\beta$, $-0.009, P = .75$).

**Discussion**

This population-based study found a reduced FAZ circularity and FD and an increased VDI in amblyopic eyes compared with control eyes as measured by swept-source OCT-A. Reduced FAZ circularity and increased VDI was associated with poorer visual acuity. This article identified new OCT-A metrics with a potential to be used as objective tools in the diagnosis and monitoring of amblyopia and its treatment in children.

Foveal avascular zone area and VD were the main parameters used in previous OCTA studies to quantify microvasculature in amblyopia, but they have been shown to be affected significantly by confounders and may not be the most sensitive markers to assess foveal health and correlate with central vision.\(^\text{17}\) We believe unitless parameters, such as FAZ circularity, VDI, and FD, represented as a ratio are more reliable and reproducible measurements to monitor structural changes in the retinal microvasculature. We did not find a difference between amblyopic and control eyes in FAZ area and VD in the superficial capillary layer. We also did not find a correlation between these 2 parameters with logMAR visual acuity. Prior studies analyzing FAZ and VD alone have given inconsistent results. Yilmaz et al\(^\text{11}\) and Lonngi et al\(^\text{12}\) reported a lower VD in the superficial and deep capillary plexus of amblyopic eyes. A recent cross-sectional study with 85 children with amblyopia with 66 age-matched controls showed reduced VD in the superficial capillary plexus only but not the deep capillary plexus.\(^\text{16}\) However, like this study, Demirayak et al\(^\text{15}\) did not find a difference in retinal capillary plexus densities between amblyopic eyes, controls, and fellow eyes of patients with unilateral amblyo-
Association of Optical Coherence Tomography Angiography Metrics With Decreased Vision in Amblyopic Eyes

Original Investigation Research

It is known that several covariates can be significantly associated with decreased vision in amblyopic eyes. In addition, it is known that several covariates can be significantly associated with decreased vision in amblyopic eyes. Association of these covariates with decreased vision in amblyopic eyes is uncertain, as reported in animal studies. Animal studies also did not reveal a difference in VD between amblyopic eyes and controls. These inconsistencies could be attributed to small sample size, heterogeneous study populations, and assessment methods. In addition, it is known that several covariates can be significantly associated with decreased vision in amblyopic eyes.

Table 3. Linear Regression Analysis Between FAZ Area, FAZ Circularity, Vessel Density, Vessel Diameter Index, and Fractal Dimension With Best-Corrected Visual Acuity After Adjusting for Covariates

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>β (95% CI)</th>
<th>Standardized β</th>
<th>P value for linear regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log FAZ area</td>
<td>−0.234 (−0.511 to 0.043)</td>
<td>−0.045</td>
<td>.09</td>
</tr>
<tr>
<td>Log FAZ circularity</td>
<td>−0.365 (−0.533 to −0.198)</td>
<td>−0.133</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Log fractal dimension</td>
<td>−0.006 (−0.019 to 0.007)</td>
<td>−0.029</td>
<td>.35</td>
</tr>
<tr>
<td>Log vessel diameter index</td>
<td>0.166 (0.069 to 0.263)</td>
<td>0.097</td>
<td>.001</td>
</tr>
<tr>
<td>Log vessel density</td>
<td>−0.002 (−0.015 to 0.011)</td>
<td>−0.009</td>
<td>.75</td>
</tr>
</tbody>
</table>

Abbreviation: FAZ, foveal avascular zone.

Strengths and Limitations

Strengths of our study include a standardized image acquisition protocol and strict definition of image artifacts for quality control in a reading center. The fully automated custom MATLAB program that we recently developed is able to quantify detailed information from the macula capillary network from OCT-A metrics. Two dedicated assessors of the images were also masked to the study participants’ initial diagnoses. Based on the results from our previous population-based study on the distributions of quantitative OCT-A metrics, we ensured accuracy of the results by adjusting for all known covariates.

Limitations of this study should be noted. We did not use a specific scoring system for artifacts, such as image tilt, for our OCT-A quality control. In the calculation of vessel density, our measurement excluded the central Early Treatment Diabetic Retinopathy Study 1-mm circle at the center but not specifically the FAZ. This reduces the effect of FAZ as an interacting variable but does not completely eliminate its effect. Furthermore, the measurement of OCT-A metrics includes retinal vessels and retinal capillaries and it does not separate arterioles and venules. Changes in OCT-A metrics may reflect changes in arterioles or venules or both. Associated with study design, only 1 eye of each patient was recruited in the study and interocular correlation cannot be commented in this study. Because of the cross-sectional nature of our data, we could only establish an association, but not a causal relation-
ship, between amblyopia and OCT-A metrics. We also did not explore specific OCT macula findings of foveal pit excavation, foveal bulge, and ganglion cell analysis in association with amblyopia, which have been previously shown to be different in ambyopic eyes.16–19 Future studies are needed to determine how these primary structural changes may affect the association with macular microvasculature as measured by OCT-A. Lastly, our study population includes a narrow racial and age band, hence the study results may not be applicable to other populations.

Conclusions

This population-based study supports the presence of morphological changes in the macular microvasculature in amblyopic eyes of children. Such changes are associated with poorer visual acuity, and we believe that OCT-A and quantitative OCT-A metrics, such as FAZ circularity, VDI, and FD, have a potential to be used as a reliable, objective, and automated tool in the diagnosis and recognition of amblyopia.

REFERENCES


5. Stryker MP, Harris WA. Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J Neurosci. 1986;6(8):2171-2173. doi:10.1523/JNEUROSCI.06-08-02171986


Amblyopia has been a diagnosis of exclusion. Besides best-corrected visual acuity, there are presently no standardized metrics that are used for the diagnosis and evaluation of amblyopia. For more than a decade, studies using structural optical coherence tomography (OCT) have identified several differences between amblyopic and control eyes. \(^1\) Qualitatively, the increase in height of the ellipsoid zone at the central fovea was noted to be attenuated or absent in 60% of amblyopic eyes compared with 29% of normal eyes. \(^2\) Several studies had reported that the foveal minimum thickness was greater in amblyopic eyes compared with normal controls, but the differences were small. \(^2,3\) Differences had also been reported in choroidal and retinal nerve fiber layer thicknesses, whereas other studies had reported no substantial differences between amblyopic and control eyes. \(^4\,5\,13\)

More recently, OCT angiography (OCT-A) has been used to study amblyopes, although various studies had reported contradictory results. While some reported decreased foveal avascular zone size \(^6\,5\,3\) and macular vascular density index, \(^4\) others reported no difference. \(^5,6\) Image quality, adjustment for age, and refractive error might explain the plurality in results. Studies on variations of OCT-A findings, including vascular density index, foveal avascular zone size, and circularity among normal cohorts, had also provided valuable insights on the influence of various patient and ocular factors on OCT-A findings, including age, sex, central subfield retinal thickness, and axial length. \(^7\)

In this issue of *JAMA Ophthalmology*, Wong et al. \(^6\) reported the findings from a case-control study nested within a large population-based study involving more than 4000 children of Chinese descent aged 6 to 8 years. To our knowledge, this is the first population-based study on amblyopia using OCT-A. The authors found that in the superficial capillary plexus, eyes with amblyopia had decreased foveal avascular zone circularity, decreased fractal dimension, and increased vessel diameter index compared with normal controls. The authors are to be commended for their methodology, including image quality assurance, standardization of image grading, and rigor of statistical analysis.

These findings are not mere scientific curiosity. OCT-A has the potential to be an important diagnostic tool for amblyopia if a clear cutoff between normal and abnormal eyes can be identified among these novel parameters. OCT-A may also serve as an objective estimate of the severity of amblyopia, especially important in cases of suspected double pathologies. However, it would be important to validate these findings in future studies, involving participants of different races/ethnicities and age ranges. Equally important is the reproducibility of these metrics across different OCT-A devices. In this respect, the authors should be commended for their focus on unitless parameters, which may be more reproducible across different OCT-A machines.

Another consideration is the convenience and ease of use for ophthalmologists in a busy clinical practice. If these measurements can be automated and made conveniently available across OCT-A devices, it may be more likely to be adopted. As the authors had correctly pointed out, these associations do not imply causality, and it is presently unclear how these findings may relate to the pathogenesis of amblyopia and at what stage they arise. Current literature on angiogenesis of the macula in utero suggests vasculature development reflects and follows the metabolic needs of neuronal development.