Thicknesses of macular inner retinal layers in children with anisometropic amblyopia

Zheren Xia
The Second Affiliated Hospital and Yuying CHildren's Hospital of Wenzhou Medical University
https://orcid.org/0000-0001-7573-8462

Hao Chen
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University

Suilian Zheng (✉ doctorlily@hotmail.com)
https://orcid.org/0000-0002-7070-6688

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Abstract

Background To investigate the thicknesses of macular inner retinal layers in children with anisometric amblyopia through spectral-domain optical coherence tomography (SD-OCT). Methods Thirty-seven children with anisometric amblyopia and fifty-seven children with normal vision participated in the study. Both eyes of children with amblyopia and the right eyes of children with normal vision underwent scanning with the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). The segmentation of retinal layers was performed automatically to measure individual inner retinal layers in the five sectors of the macula. An independent-sample t-test was used to compare measurements of anisometric eyes and fellow eyes with control eyes. Results There was no significant difference in the total macular thickness in the five sectors between amblyopic and control eyes. However, in the peripheral macular area, three of the four quadrants of both the ganglion cell layer (GCL) and the inner plexiform layer (IPL) thickness were significantly reduced in amblyopic eyes than in control eyes. Moreover, two of the four quadrants of the GCL thickness and three of the four quadrants of the IPL thickness in the peripheral macular area were significantly reduced in fellow eyes than in control eyes. Conclusions The SD-OCT data revealed differences in the thicknesses of some macular inner retinal layers in both eyes of children with anisometric amblyopia compared with those with emmetropia, indicating that structural changes might exist in the retina of children with amblyopia.

Background

Amblyopia is one of the most common diseases of monocular visual impairment in children. The main causes include strabismus, anisometropia, or an obstruction along the visual axis. In the study of the pathogenesis of amblyopia, it has been generally believed that amblyopia is a pathophysiological change from retinal ganglion cells to the visual cortex, including two theories: central [1, 2] and peripheral [3, 4]. Animal experiments and functional magnetic resonance imaging have confirmed the presence of histological changes in the hypothalamus in patients with amblyopia [1, 5]. However, because of technological limitations of assessment, the change of retinal structure is still controversial.

The Spectralis OCT (Heidelberg, Germany), based on spectral-domain optical coherence tomography (SD-OCT) technology, has improved resolution that is capable of performing more precise measurements of retinal layers. Furthermore, it can provide automated segmentation and quantification of each retinal layer with a specific software. Thus, investigators began to explore the changes in OCT images to verify the presence of retinal dysfunction. The changes of the inner retina, such as the ganglion cell layer (GCL) and retinal nerve fiber layer (RNFL), are applied to diagnose glaucoma, multiple sclerosis, anterior ischemic optic neuropathy, and other diseases [6–8].

This study aimed to assess alterations in the thicknesses of macular inner retinal layers in children with anisometric amblyopia using the Spectralis OCT.

Methods
Thirty-seven children with anisometropic amblyopia who did not receive treatment were recruited from July 2014 to February 2017 in the Ophthalmology Department, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. Both eyes of each child were included in the study, with the contralateral eye as the fellow eye group. The right eyes of 57 children with emmetropia were enrolled as control subjects (Table 1).

All subjects were aged from 4 to 14 years. The amblyopic subjects were enrolled in this study with the best-corrected visual acuity (BCVA) between 20/32 and 20/400 in the amblyopic eye and 20/40 or better in the normal eye, respectively. The intereye BCVA difference is more than 2 logarithm of the minimum angle of resolution lines (logMAR). Anisometropia was defined as the difference of interocular spherical equivalent or astigmatism equal to or more than 1.5 diopter (D). The control subjects should have refractive error ranging from −0.50 to +0.50 DS and visual acuity equal to or greater than 20/20 in each eye. Patients were excluded if they were not cooperative enough for OCT examination or combined with several conditions as follows: organic eye diseases such as cataract, glaucoma, retinal diseases; strabismus; a history of intraocular surgery.

All subjects underwent a full ophthalmic examination, including visual acuity, extraocular movements, manifest refraction, slit-lamp biomicroscopy, intraocular pressure, fundus examination, cycloplegic refraction, and axial length (using Carl Zeiss IOL Master; Carl Zeiss AG, Oberkochen, Germany).

All subjects gave their informed consent according to the Declaration of Helsinki (1964), and the study was approved by the Research Ethics Committee of The Second Hospital of Wenzhou Medical University. Informed consent was obtained from all individual participants (or legal parent or guardian for children) included in the study.

Fig. 1 The Early Treatment Diabetic Retinopathy Study (ETDRS) grid, showing C1, T3, I3, N3, and T3

Fig. 2 Retinal layer analysis performed by the segmentation software of the Spectralis

The thickness of each layer was identified and measured automatically by Spectralis OCT ((Heidelberg Engineering, Heidelberg, Germany)). The TruTrack active eye-tracking system was applied to increase scan quality. Volume scan mode was performed to obtain a 20° x 20° macular cube scan image (49 B-scan sections, 120 μm spacing, and 512 A scans/B scans). Macular thickness was measured in nine quadrants, using the Early Treatment Diabetic Retinopathy Study (ETDRS) grid, which comprises three concentric circles with diameters of 1, 3, and 6 mm. For the reason of children's cooperation, we took the area only within the inner ring for analysis in order to ensure the reliability of the data. The parameters in the following five sectors were recorded in this study: C1 (the average thickness in the central 1 mm diameter) and N3/I3/T3/S3 (the average thickness in the nasal/inferior/temporal/superior quadrant of a concentric ring, with an inner diameter of 1 mm and outer diameter of 3 mm of the ETDRS grid; Fig. 1).

The retina was segmented automatically into seven layers with a built-in software for the Spectralis OCT: (1) retinal nerve fiber layer (RNFL), (2) ganglion cell layer (GCL), (3) inner plexiform layer (IPL), (4) inner
nuclear layer (INL), (5) outer plexiform layer (OPL), (6) outer nuclear layer (ONL), and (7) retinal pigment epithelium (RPE). From the map of retinal layer thickness, data were divided into five macular sectors. Each individual layer thicknesses of these five sectors were registered. We chose the four inner retinal layers (RNFL, GCL, IPL, and INL) for analysis (Fig. 2).

SPSS software version 22.0 (International Business Machines Corp., NY, USA) was applied for the statistical analysis of our data. Continuous data has been presented as the means ± standard deviations (SD). The normal distribution of continuous variables was assessed by Kolmogorov–Smirnov test. Snellen visual acuities were transformed to logMAR visual acuities for the statistical analysis. Independent-sample *t*-test was used to compare refractive error, axial length, and mean layer thicknesses of anisometric eyes and fellow eyes with control eyes respectively. Meanwhile, the same statistical method was used to compare the ages between the anisometric subjects and the control subjects. *P*-values <0.05 were considered statistically significant.

**Results**

The study included 37 children with anisometric amblyopia (21 females, 16 males), with a mean age of 7.43 ± 2.62 (range: 4–14) years. Of these children, 16 had amblyopia in their right eye and 21 had it in their left eye. No significant difference was found in age between the amblyopic subjects and controls (*p* = 0.19). The mean spherical equivalent refractive error was +1.52 ± 3.42 (range: −4.38 to +7.50) D in amblyopic eyes and +0.21 ± 1.50 (range: −3.63 to +3.50) D in fellow eyes. There was a significant difference in the refractive error between amblyopic and control eyes (*p* = 0.01, 0.35), whereas no significant difference was noted between amblyopic and fellow eyes. Both amblyopic and fellow eyes had no significant difference in the axial length of control eyes (*p* = 0.08, 0.12). The mean BCVA in logMAR was 0.40 ± 0.22 in amblyopic eyes, which was significantly different in control eyes (*p* < 0.01).

The total macular thickness (TMT) in C1 was 266.90 ± 23.22 µm in amblyopic eyes, 263.90 ± 22.84 µm in fellow eyes, and 255.91 ± 18.87 µm in control eyes. There was no significant difference in TMT in the five sectors between amblyopic and control eyes, and only in T3, there was a significant difference between fellow and control eyes (*p* = 0.04; Table 2).

The macular RNFL thickness did not differ significantly in the five sectors between amblyopic and control eyes. The GCL thickness measurements in S3, T3, and N3 were significantly reduced between amblyopic and fellow eyes. All measurements, except C1 and T3 of the IPL thickness, were significantly reduced in amblyopic eyes compared with control eyes. There was a significant difference only in C1 in the INL thickness measurements between amblyopic and control eyes (Table 3).

No significant differences were noted between fellow and control eyes in the RNFL and INL thicknesses. The GCL thickness measurements in S3 and T3 were significantly reduced in fellow eyes. The IPL thickness measurements in S3, T3, and N3 were significantly decreased in fellow eyes as compared to control eyes (Table 3).
All the findings and \( p \)-values are summarized in Tables 2 and 3.

**Discussion**

Bilateral alterations of inner retinal thickness in children with anisometropic amblyopia was found according to our study. TMT in anisometropic amblyopic eyes measured by the Spectralis has no difference with that in normal eyes. However, three areas of the GCL and IPL thicknesses of anisometropic amblyopic eyes were much smaller than those of control eyes.

Several studies have examined macular thickness with OCT in amblyopia in the past decade. In this study, there was no significant difference noted in TMT between amblyopic and control eyes. Similar findings were reported in some studies [4, 9, 10], whereas Yi et al. [3] reported that the fovea of amblyopic eye tend to be thicker than that of the normal fellow eye, while the inner and outer macula tend to be thinner. The difference in the results of these studies may be due to the experimental design, ethnicity, or refractive error. Furthermore, many of these studies were performed with time-domain OCT (TD-OCT), which has a resolution of 10 \( \mu \)m axially. In addition, most of the differences in thicknesses reported were even less than 10 \( \mu \)m, which made the outcomes of these studies unreliable. Besides, because of the low resolution of TD-OCT, these studies were limited to TMT.

In recent years, with a wide application of SD-OCT, it is possible to analyze the macular area on the thicknesses of individual layers. Some previous studies have analyzed the macular area in children with amblyopia. Chen et al. [11] analyzed the thickness of each retinal layer at the foveal center and 0.5 mm from the foveal center in four directions. They found that the nasal nerve fiber and inferior inner nuclear layers in amblyopic eyes were significantly thicker than those in control eyes. Kyung-Ah et al. [12] evaluated the thickness of the central and peripheral macular areas of eyes with unilateral amblyopia; they found significant thinning of GCL and IPL in amblyopic eyes. The current study had similar results with them. In the peripheral macular area of amblyopic eyes, three of the four quadrants of both GCL and IPL thicknesses were significantly reduced, indicating that structural differences might exist in GCL and IPL in amblyopic eyes.

IPL is the second synaptic layer of the retina. The synapses in bipolar, amacrine, and ganglion cells in IPL are involved in the construction of a complex visual signal–processing network. Ji et al. [13] found that the P1 wave amplitude density of mfERG first-order kernel in amblyopic eyes was significantly attenuated compared with that in control eyes, which may reflect the abnormality of the retinal nerve in bipolar cell function and visual information transmission. Although the relationship between the retinal structure and function of amblyopia remains a question, Betul et al. [14] reported a significant reduction of PERG amplitude in amblyopic eyes was found when compared with normal eyes. Whereas no significant relationship between OCT and PERG parameters was discovered. Thus, further research is required to clarify whether or not the structural changes existing in GCL and IPL lead to functional visual loss in amblyopia.
In the current study, two of the four quadrants of the GCL thickness and three of the four quadrants of the IPL thickness in the peripheral macular area were significantly decreased in fellow eyes as compared to control eyes. This is consistent with some studies [15, 16] that reported both eyes in children with unilateral amblyopia had deviations when compared with normal children. Chang-Bing et al. [17] reported that anisometropic amblyopia resulted in both monocular and interocular dysfunction. This functional imbalance between amblyopic eye and fellow eye may cause irreversible changes which affect the visual pathway of both eyes. This is also the reason why we compared amblyopic eyes with normal eyes instead of fellow eyes.

Unlike some previous studies, we adopted the built-in automatic layer segmentation software of the Spectralis OCT, which made it possible to compare the results of different laboratories with the same platform. Besides, this study analyzed the average thickness data of each area of the macula to avoid the possible deviation caused by measuring only one point in each direction in some studies [11, 12].

Limitations of our study should be discussed: The sample size of anisometropic amblyopic eyes was relatively small, though it was bigger than that in previous studies. Besides, for the reason of children's cooperation, the thickness of each layer in the outer ring was not analyzed in order to ensure the reliability of the data.

**Conclusions**

The SD-OCT data revealed differences in the thicknesses of some macular inner retinal layers in both eyes of children with anisometropic amblyopia compared with those with emmetropia, indicating that structural changes might exist in the retina of children with amblyopia. Further studies, including a larger area in the macula, with more numbers of patients and correlation with retinal function are required to confirm these findings.

**Abbreviations**

BCVA: Best-corrected visual acuity; GCL: Ganglion cell layer; INL: Inner nuclear layer; IPL: Inner plexiform layer; logMAR: Logarithm of the minimum angle of resolution; ONL: Outer nuclear layer; OPL: Outer plexiform layer; RNFL: Retinal never fiber layer; RPE: Retinal pigment epithelium; SD-OCT: Spectral-domain optical coherence tomography; TD-OCT: time-domain OCT; TMT: The total macular thickness.

**Declarations**

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on request.

Authors’ contributions

ZX and SZ designed and conducted the study; ZX and HC collected, analyzed, and interpreted the data collected; ZX prepared the manuscript; and SZ critically reviewed and approved the final draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee, The Second Hospital of Wenzhou Medical University, Wenzhou, China. All procedures performed during the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki (1964) and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants (or legal parent or guardian for children) included in the study.

Consent for publication

Informed consent was obtained from all individual participants (or legal parent or guardian for children) to publish the study.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Demographic and clinical description

| Group          | n   | Male/Female | Age (mean ± SD) | Axial length (mm) | LogMAR (D) | Refractive error (D) |
|----------------|-----|-------------|-----------------|-------------------|------------|---------------------|
| Amblyopic group| 37  | 16/21       | 7.43 ± 2.62     | 22.41 ± 1.76      | 0.40 ± 0.24 | +1.52 ± 3.42        |
| Fellow group   | 37  | 16/21       | 7.43 ± 2.62     | 22.57 ± 1.39      | 0.06 ± 0.08 | +0.21 ± 1.50        |
| Control group  | 57  | 25/32       | 8.05 ± 1.33     | 22.96 ± 0.67      | −0.02 ± 0.03 | −0.02 ± 0.16      |

LogMAR logarithm of the minimum angle of resolution

Table 2 The mean values of the total macular thickness (in μm)

|                 | Amblyopic eyes (mean ± SD) | Fellow eyes (mean ± SD) | Control eyes (mean ± SD) | P1   | P2   |
|----------------|-----------------------------|-------------------------|--------------------------|------|------|
| C1             | 254.83 ± 23.73              | 253.48 ± 21.51          | 255.91 ± 18.87           | 0.81 | 0.56 |
| S3             | 339.64 ± 16.70              | 344.86 ± 28.21          | 342.84 ± 12.73           | 0.3  | 0.63 |
| I3             | 330.81 ± 19.66              | 335.78 ± 20.98          | 335.71 ± 13.01           | 0.15 | 0.98 |
| T3             | 322.72 ± 18.59              | 321.54 ± 15.89          | 327.52 ± 12.35           | 0.14 | 0.04a|
| N3             | 333.11 ± 24.19              | 336.27 ± 16.48          | 340.70 ± 14.13           | 0.06 | 0.17 |

P1 amblyopic eyes vs control eyes, P2 fellow eyes vs control eyes, SD standard deviation

A statistically significant difference between (p ≤ 0.05)

Table 3 The mean values of individual inner retinal layer thickness (in μm)
|                | Amblyopic eyes (mean ± SD) | Fellow eyes (mean ± SD) | Control eyes (mean ± SD) | P1   | P2   |
|----------------|----------------------------|-------------------------|--------------------------|------|------|
| C1 RNFL        | 11.54 ± 3.00               | 11.00 ± 2.52            | 11.11 ± 2.33             | 0.46 | 0.84 |
| S3 RNFL        | 24.05 ± 3.37               | 25.84 ± 10.38           | 23.02 ± 2.86             | 0.11 | 0.12 |
| I3 RNFL        | 23.35 ± 3.42               | 26.38 ± 23.65           | 23.44 ± 2.78             | 0.89 | 0.46 |
| T3 RNFL        | 17.30 ± 2.47               | 16.65 ± 1.25            | 16.60 ± 1.29             | 0.08 | 0.85 |
| N3 RNFL        | 20.11 ± 2.87               | 20.03 ± 3.35            | 19.88 ± 2.32             | 0.67 | 0.80 |
| C1 GCL         | 15.24 ± 6.26               | 14.27 ± 7.01            | 14.05 ± 3.91             | 0.26 | 0.85 |
| S3 GCL         | 51.43 ± 4.11               | 51.46 ± 4.60            | 54.53 ± 4.14             | <0.01a | <0.01a |
| I3 GCL         | 50.30 ± 5.58               | 51.16 ± 4.66            | 51.74 ± 7.50             | 0.32 | 0.65 |
| T3 GCL         | 46.16 ± 4.43               | 45.24 ± 5.55            | 49.18 ± 3.55             | <0.01a | <0.01a |
| N3 GCL         | 48.19 ± 6.57               | 49.73 ± 5.36            | 51.56 ± 4.00             | 0.01a | 0.06a |
| C1 IPL         | 19.27 ± 4.95               | 19.46 ± 5.02            | 19.02 ± 3.19             | 0.76 | 0.60 |
| S3 IPL         | 40.11 ± 2.32               | 40.30 ± 3.10            | 41.54 ± 2.44             | 0.01a | 0.03a |
| I3 IPL         | 39.38 ± 3.62               | 40.19 ± 2.42            | 40.88 ± 2.47             | 0.02a | 0.19 |
| T3 IPL         | 39.73 ± 3.54               | 39.22 ± 3.89            | 40.72 ± 2.30             | 0.10 | 0.04a |
| N3 IPL         | 39.62 ± 4.01               | 40.70 ± 3.16            | 42.16 ± 2.94             | <0.01a | 0.03a |
| C1 INL         | 17.78 ± 5.50               | 16.65 ± 5.43            | 15.63 ± 4.45             | 0.04a | 0.32 |
| S3 INL         | 41.41 ± 4.16               | 41.43 ± 6.47            | 41.42 ± 2.97             | 0.98 | 0.99 |
| I3 INL         | 41.57 ± 5.17               | 41.43 ± 4.61            | 40.88 ± 3.60             | 0.48 | 0.52 |
| T3 INL         | 38.81 ± 4.88               | 37.76 ± 4.63            | 38.96 ± 3.15             | 0.87 | 0.17 |
| N3 INL         | 40.54 ± 5.13               | 41.54 ± 5.54            | 39.98 ± 3.70             | 0.54 | 0.11 |

*P1* amblyopic eyes vs control eyes, *P2* fellow eyes vs control eyes, *SD* standard deviation

aA indicates statistically significant difference between (*p* ≤ 0.05)

**Figures**
Figure 1

The Early Treatment Diabetic Retinopathy Study (ETDRS) grid, showing C1, T3, I3, N3, and T3
Figure 2

Retinal layer analysis performed by the segmentation software of the Spectralis