Original Article

Quantitative Analysis of Inner, Middle, and Outer Retinal Thickness by Optical Coherence Tomography in Children and Adolescents

Fariba Ghassemi[1,2], Farhad Salari[1], Vahid Hatami[1], Fatemeh Bazvand[1,2], Hadi Shamouli[1], Masoumeh Mohebbi[1,3], Siamak Sabour[4,5]

[1]Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran, [2]Retina and Vitreous Service, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran, [3]Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran, [4]Department of Clinical Epidemiology, School of Health and Safety, Safety Promotion and Injury Prevention Research Center, Tehran, Iran, [5]Department of Clinical Epidemiology, School of Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Purpose: To describe the variance of inner, middle, and outer retinal layer thicknesses (IRT, MRT, and ORT) at the macular area in children and adolescents with normal eyes in different age groups.

Methods: This cross-sectional study enrolled subjects aged 5–18 years with normal eyes. The macula was scanned by optical coherence tomography (6 mm × 6 mm AngioScan-Optovue). Four age groups were defined (≤7, 7–10, 11–14, and ≥14 years). The influences of age and gender were analyzed.

Results: One hundred and thirty-nine eyes of 69 subjects with a mean age of 10.92 ± 3.51 years were registered. The mean whole macular thickness (MT) was 297.32 ± 11.05 in males and 303.197 ± 13.32 in females ($P = 0.01$, t-test). The MT in each aging group was 301.47 ± 2.5, 295.53 ± 1.71, 300.81 ± 2.12, and 298.6 ± 1.87, subsequently ($P = 0.17$, analysis of variance test). Significant differences were found between the sexes at the perifoveal area and mainly in IRT. No correlation between eyes was noted. We observed that the RT fluctuates during growth and that gender has some influence on the evolution of RT. IRT and MRT changed reciprocally in all macular areas, whereas ORT expanded in all age groups of children and adolescents.

Conclusions: No subsegmental retinal thickness difference between eyes was observed in pediatric groups in this study, while gender had some influence on perifoveal IRT. Despite the fact that this study is not a longitudinal study, we can get some insight into the developmental changes in retinal thickness and its clinical applications in children and adolescents.

Keywords: Adolescents, Central macular thickness, Children, Inner retinal thicknesses, Middle retinal thicknesses, Outer retinal thicknesses, Spectral domain optical coherence tomography

Address for correspondence: Fariba Ghassemi, Eye Research Center, Farabi Eye Hospital, Qazvin Square, Tehran, Iran.
E-mail: fariba.ghassemi@gmail.com
Submitted: 18-Jul-2023; **Revised:** 25-Aug-2023; **Accepted:** 26-Aug-2023; **Published:** 21-Dec-2023

Introduction

The macula is an oval-shaped region of the human retina with a diameter of roughly 5.5 mm that contains the foveal, parafoveal, and perifoveal retinas.[1] According to earlier research, the maturation of the human fovea is a long and complex developmental process that begins about midgestation and lasts until around 13 years of age.[2] Understanding the mechanisms that contribute to the development of the central retina is critical in the treatment of congenital and acquired ocular diseases that can impair vision during childhood, adolescence, and later in life.[3-5] With the introduction of high-resolution imaging utilizing

Access this article online

Quick Response Code:

Website: https://journals.lww.com/joco

DOI: 10.4103/joco.joco_141_23

How to cite this article: Ghassemi F, Salari F, Hatami V, Bazvand F, Shamouli H, Mohebbi M, *et al*. Quantitative analysis of inner, middle, and outer retinal thickness by optical coherence tomography in children and adolescents. J Curr Ophthalmol 2023;35:182-9.

optical coherence tomography (OCT), we now have a better knowledge of normal and pathological retinal development. The technique allows *in vivo* cross-sectional visualization of biological tissue at micrometer resolution of the retina, retinal nerve fiber layer, and optic nerve head.[6] To evaluate pathological changes, it is important to quantify normal retinal thickness.[7] In addition, to make an accurate diagnosis of a kid's retinal disease, quantitative measures from the child should be compared to age-matched normal controls. There are few reported normal values from children, and only normative studies of Turkish and American children are accessible in the literature.[8,9] The current study uses the spectral domain OCT (SD-OCT) to create a normative pediatric database for inner, middle, and outer retinal layer thicknesses (IRT, MRT, and ORT) at the macular areas in healthy Iranian children and adolescents.

Methods

The study was conducted on healthy children and adolescents who came to Farabi Eye Hospital, Tehran, Iran, for routine eye examinations and students from two schools in Tehran who were invited to participate in the experiment from April 2018 to May 2020. An institutional review board approval was obtained for this cross-sectional study from the Farabi Eye Hospital, Department of Ophthalmology, Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.1054). The tenets of Declaration of Helsinki were followed during the study. Participants provided written informed consent. Fifteen eligible children or their parents did not provide informed consent and were thus removed from the study. Patients' data were anonymized before data analysis.

The study included healthy children and adolescents with best-corrected visual acuity of 20/20, a refractive error between −1 and +1 diopters, and intraocular pressure of <21 mmHg. Exclusion criteria were any systemic and ocular disease, any positive past medical, surgical, and drug history.

The patients were divided into four groups based on their age: ≤7, 7–10, 11–14, and 14–18. Imaging and examinations were performed on the same day during working hours (up to 2 PM). On the Optovue RTVue XR Avanti (Optovue Inc., Freemont, California, USA), we obtained OCT angiography images using the split-spectrum amplitude-decorrelation angiography algorithm. A 40 nm wavelength laser provides 70,000 A-scans per second; 304 A-scans form a B-scan, while 304 vertical and horizontal lines were tested in the scanning area to obtain a three-dimensional data cube and eliminate motion artifacts. Volume scans by 3 mm × 3 mm and 6 mm × 6 mm centered onto the fovea were performed in both eyes for each patient using 400 raster lines.

The inbuilt software defined the thickness of the inner retina between the internal limiting membrane (ILM) and the outer boundary of the inner plexiform layer (IPL) (IRT, ILM-IPL), the middle retina from the outer boundary of IPL to the outer boundary of outer plexiform layer (OPL) (MRT, IPL-OPL) and thickness of the outer retina as outer margin of OPL to the outer boundary of retinal pigmentary epithelium (ORT, OPL-BRM). In this study, the thickness was calculated for different sectors (whole image, central 6 mm diameter at fovea, parafovea, perifovea, and superior and inferior half of the extrafoveal areas) based on the Early Treatment Diabetic Retinopathy Study grid. OCT automatically locates the fovea. A 1.0 mm diameter circle was defined as the foveal region. The parafoveal area was defined as a ring centered on the foveal region with an inner diameter of 1.0 mm and an outer diameter of 3.0 mm. The perifoveal area was defined as a ring centered on the foveal region with an inner diameter of 3.0 mm and an outer diameter of 6.0 mm. The retinal thickness measurements were generated through a 21-line raster scanning protocol that was designed to allow frame averaging to maximize scan quality in this sample of children.

For statistical analysis, the thickness of foveal, parafoveal, perifoveal, and whole 6 mm diameter (WI) and superior (sup) and inferior (inf) half (hemi) data of parafovea and perifovea area from 6 mm × 6 mm images volumetric scans were composed. When two eyes from the same subject were eligible (in terms of the quality of the images), both eyes were included in the analysis. Two ophthalmologists (FG and VH) evaluated the quality and segmentations of OCT images.

Statistical analysis

The sample size was calculated as 97 eyes with a 95% confidence interval, 10% of the vascular density unit change (per percentage-quantitative data), and 50% population proportion. After determining the normality of the distribution with the Kolmogorov–Smirnov test and histogram, all quantitative data were given as mean with standard deviation. The variables with no Gaussian distribution have been reported as median with the range. Statistical software (SPSS software Version 21; SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. Inter-eye correlation was evaluated to see any significant correlation [Supplementary Table 1]. For nonparametric and parametric comparisons, the Kruskal–Wallis test and one-way analysis of variance (ANOVA) were used. *P* values were adjusted for multiple comparisons using the Bonferroni method. Two-way ANOVA on means of retinal thickness was performed to test the main effect and interaction of gender and age.

Results

Of the 85 initially examined participants, 16 had to be excluded for further investigation due to poor image quality. A total of 131 eyes of 69 individuals were investigated in this study. The mean age of all subjects was 10.92 ± 3.51 years (range, 5–18 years). 70.2% of participants (92 eyes) were male with a mean age of 10.92 ± 3.71 years, and 29.8% (40 eyes) were female with a mean age of 10.9 ± 3.03 years old. Its mean weight was 39.2 ± 15.4 kg and its mean height was 137.8 ± 21.7 cm. In each of the four groups, 26 eyes (19.9%) were in the group under 7 years, 35 eyes (26.7%) were in the group between 7 and 10 years, 43 eyes (32.8%) were in the

Table 1: Results of two-way analysis of variance analysis for evaluating the effect of age and sex and their interaction on retinal thickness in childhood per micrometer

Variable	Model	Significant	Partial η^2
The mean of whole macular thickness (0–6 mm)	Age	0.11	0.05
	Gender	0.04	0.03
	Age × gender	0.11	0.05
The mean of fovea thickness (0–1 mm)	Age	0.17	0.04
	Gender	0.32	0.01
	Age × gender	0.88	0.01
Mean of parafovea thickness (1–3 mm)	Age	0.14	0.04
	Gender	0.28	0.01
	Age × gender	0.29	0.03
Mean of perifovea thickness (3–6 mm)	Age	0.04	0.07
	Gender	0.01	0.05
	Age × gender	0.09	0.05
Mean IRT of the whole image	Age	0.01	0.09
	Gender	<0.001	0.09
	Age × gender	0.02	0.08
Mean MRT of the whole image	Age	0.03	0.07
	Gender	0.15	0.02
	Age × gender	0.11	0.05
Mean ORT of the whole image	Age	0.29	0.03
	Gender	0.95	0.02
	Age × gender	0.31	0.03

P<0.007 was considered statistically significant (Bonferroni adjustment). IRT: Inner retinal thickness, MRT: Middle retinal thickness, ORT: Outer retinal thickness

group from 11 to 14 years, and 27 eyes (20.6%) were in the group over 14 years.

The mean whole macular thickness (MT) (0–6 mm) was 299.07 ± 12.02 µm. MT was 297.32 ± 11.05 in males and 303.197 ± 13.32 in females (P = 0.01, t-test). The mean whole MT in each aging group was 301.47 ± 2.5, 295.53 ± 1.71, 300.81 ± 2.12, and 298.6 ± 1.87, subsequently (P = 0.17, ANOVA test).

The result of two-way ANOVA showed a marginally insignificant effect of gender on the whole MT [P = 0.04, Table 1]. However, The MT was not affected by age [P = 0.1, Table 1]. The course of alteration in thickness of inner, mid, and outer retina during childhood for both genders is illustrated in Figure 1a.

The mean central foveal thickness (CFT) was 251.10 µm, with the male having a thicker CFT than the female (252.15 vs. 248.61 µm, P = 0.47, Mann–Whitney U test). The median CFT in each aging group was 245.8 (236.5–253.6), 248.3 (234–257.6), 250.7 (238.7–267.2), and 251 (240.1–274.1) µm, subsequently (P = 0.2, Kruskal–Wallis) [Table 1].

Results of the two-way ANOVA on macular regions showed that the difference in CFT among different age groups was nonsignificant [P = 0.17, Table 1]. Furthermore, the CFT was not significantly affected by gender [two-way ANOVA P = 0.31, Table 1]. The course of alteration in thickness of inner, mid, and outer foveal thickness during childhood for both genders is reported in Tables 2-4 [Figure 1b].

The mean parafoveal thickness (PaFT) (1–3 mm) was 328.77 ± 12.89 µm. PaFT in males was 327.6 ± 11.75 and 331.5 ± 2.41 in females (P = 0.11, t-test). The PaFT in each aging group was 328.69 ± 12.6, 324.96 ± 11.34, 330.34 ± 15.21 and 331.3 ± 10.34, subsequently (P = 0.19, one-way ANOVA).

The two-way ANOVA showed that age [P = 0.14, Table 1] and sex [P = 0.27, Table 1] did not affect PaFT, with no significant interaction between age and sex on PaFT [P = 0.28, Table 1]. The course of alteration in thickness of inner, mid, and outer PaFT during childhood for both genders is illustrated in Figure 1c.

The mean perifoveal thickness (PeFT) (3–6 mm) was 291.75 ± 12.20 µm. PeFT was 289.58 ± 10.9 in males and 296.79 ± 13.6 in females (P = 0.01, t-test). PeFT in each aging group was 295.4 ± 13.51, 324.96 ± 11.34, 330.34 ± 15.21, and 331.3 ± 10.34, subsequently (P = 0.06, one-way ANOVA).

In the perifovea, the two-way ANOVA showed a marginally insignificant effect of age on PeFT [P = 0.03, Table 1]. However, the effect of gender was significant [P = 0.009, Table 1]. However, the PeFT was not affected by the interaction between age and sex [P = 0.09, Table 1]. The difference between the PeFT of females and males increased after 7 years old and became significant between 11 and 14 years old (mean PeFT 303.7 ± 12.1 in females and 288.9 ± 12.4 in males) [Figure 1d]. However, the difference reduced afterward and became nonsignificant after 14 years old [Figure 1d]. The course of alteration in thickness of inner, mid, and outer PeFT during childhood for both genders is illustrated in Figure 1d.

We performed the entire analysis considering one eye per case, and the results were alike. There was no significant correlation between the right and left eyes in our study for any of the study parameters (all P > 0.05) [Supplementary Table 1]. The thickness correlation coefficient for both eyes was less than 0.3 in all studied parameters [Supplementary Table 1].

Discussion

This is the first study to compare automated layered retina thickness analysis of IRT, MRT, and ORT using Optovue OCT macular segmentation software in normal children and adolescents. The mean central macular thickness (CMT) in children and adolescents aged 5–18 years of age was 251.10 ± 20.54 µm. This value was 211.39 µm for Turkish children aged 6–16 years,[9] and is thinner than results acquired from healthy adults.[10] Although CMT increased during childhood, this increase did not reach a significant statistical difference. There was no significant correlation between CMT and age in some studies.[11]

Concordant with other studies, we found that gender had some influence on total RT at the central 6 mm of the retina, with females having less CMT [Figure 1b and c].[9,12] Yanni *et al*. and

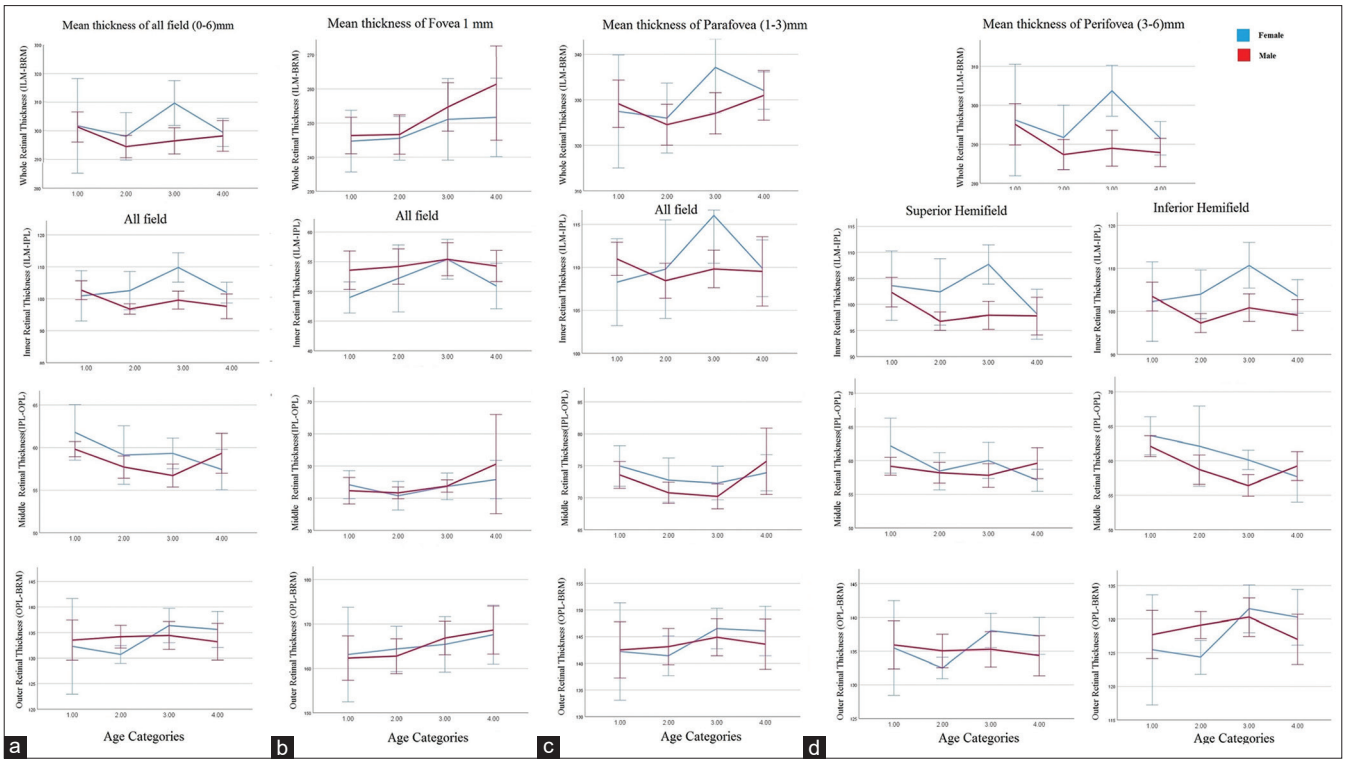

Figure 1: Alterations in retinal thickness of normal healthy children in both genders in different age groups. (a) This column shows how whole macular thickness varies from <7 years old to 18 years old in both genders. The first image illustrates changes in whole retinal thickness. The second to fourth images depict changes in inner, middle, and outer retinal thickness; (b) This column shows how foveal thickness varies during childhood. The first image illustrates changes in whole retinal thickness. The second to fourth images depict changes in inner, middle, and outer retinal thickness; (c) This column shows how parafovea thickness varies from less than 7 years old to 18 years old in both genders. The first image illustrates changes in whole retinal thickness during growth. The second to fourth images depict changes in inner, middle, and outer retinal thickness separately for girls and boys. (d) This column shows how perifoveal thickness varies from <7 years old to 18 years old in both genders. The first image illustrates changes in the whole retinal. The second to fourth images depict changes in inner, middle, and outer retinal thickness. The error bars show 95% confidence intervals

Bafiq *et al*. revealed similar results, with no significant gender differences in CMT.[8,13] According to these discrepancies, race or ethnicity appears to have an impact on CMT.[8,14] El-Dairi *et al*. recorded greater values for IRT and CFT in White children compared with Black children.[15] Huynh *et al*. found that white children had more CMT and IRT than East Asians, while Middle Eastern children had greater outer temporal MT than whites.[16]

According to our study, in the central 6 mm of the retina, gender has some impact on the course of RT alterations in the age groups [Table 1 and Figure 1a]. The difference between girls and boys was more prominent in the IRT of the perifoveal area [Figure 1d]. Interestingly, like Grover *et al*., considering all RT (ILM-BRM) segmentally, we found that the perifoveal area was thicker in females.[17] Furthermore, we showed that the RT decreased slightly from <7 years old up to 7 years old. In this period, there was no significant difference between girls and boys. RT then increased from 7 years to 14 years. This increase appeared as a higher slope in the graph of RT changes in the girls and was more prominent in the perifoveal area. This abrupt increase led to significant RT differences between males and females. After 14 years old, RT in boys continued to increase; however, in girls, RT declined suddenly and reached its pregrowth values.

In layer analysis, while in boys, the IRT decreased significantly from <7 years old to 14 years old, the IRT increased in girls in the same period and peaked at 14 years old [Figure 1b]. In the same period, the MRT in both sexes underwent thinning. This thinning in girls was significant only in the inferior hemifield. Our data revealed that ORT in boys remained stable during childhood; however, the outer retina in girls grew significantly from 7 to 14 years old.

Puberty starts from 8 to 12 years old in girls and 9–14 years in boys.[18] During this period, a variety of hormonal changes leads to significant somatic changes in humans.[19] As the observed changes in RT occur during puberty and vary significantly between males and females, we hypothesized that this retinal alteration could be due to sexual hormonal changes. Sex hormones have been shown to have a neuroprotective effect in normal retinas.[20] Further animal and human studies are required to evaluate the role of gonadal hormones on the physiology and pathology of the retina. Previous studies demonstrated the gonadal hormone receptors are distributed unevenly throughout the retina.[21] This study showed that in the middle retina, the inferior hemifield was more affected during puberty, while in the outer retina, the superior hemifield was affected by puberty. Further study is required to evaluate variation in

Table 2: Inner retinal thickness of normal children and adolescents in different age groups presented separately for both sexes per micrometer

	≤7	8–10	11–14	>14	Significant	*Post-hoc* tests
Fovea						
Female	48.99±1.31	52.19±2.82	55.42±1.68	50.91±1.92	0.18	
Male	53.57±1.62	54.19±1.49	55.41±1.39	54.29±1.43	0.83	
Parafovea (superior-hemifield)						
Female	108.63±2.37	109.6±2.84	115.48±2.77	109.1±1.83	0.19	
Male	110.3 (107.8–113.1)	109 (105.4–111.6)	110 (104.7–113.5)	106.7 (102.3–117.8)	0.54	
Parafovea (inferior-hemifield)						
Female	107.94±2.73	109.95±2.94	116.6±2.82	110.69±1.56	0.13	
Male	109.9 (107.7–114.8)	109.1 (106.6–113.1)	111.5 (104.6–115.3)	108.3 (105.4–118.3)	0.54	
Perifovea (temporal)						
Female	89.51±2.87	87.22±2.4	91.81±2.12	84.44±1.82	0.14	
Male	90.28±1.16	84.38±0.74	86.27±1.17	87.33±1.74	0.01	≤7 versus 8-10, *P*=0.004
Perifovea (superior)						
Female	103.96±3.84	102.27±3.24	107.11±1.66	98.1±2.92	0.13	
Male	102.13±1.55	95.9±1.01	96.68±1.46	95.89±2.09	0.02	≤7 versus 8-10, *P*=0.02
Perifovea (nasal)						
Female	118.81±3.34	122.56±3.52	128.6±2.84	118.49±2.37	0.07	
Male	117.96±1.66	112.48±1.04	115.29±1.64	116.07±2.28	0.15	
Perifovea (inferior)						
Female	99.73±4.4	100.83±3.33	109.13±2.37	102.15±2.19	0.09	
Male	101.12±1.92	95.33±1.34	99.25±1.69	96.08±1.68	0.05	
Perifovea (superior-hemifield)						
Female	103.63±3.33	102.41±3.19	107.69±1.89	98.13±2.41	0.08	
Male	102.33±1.42	96.78±0.87	97.89±1.34	98.29±1.98	0.04	≤7 versus 8-10, *P*=0.03
Perifovea (inferior-hemifield)						
Female	102.24±3.76	103.96±2.51	110.7±2.47	103.48±1.65	0.08	
Male	103.44±1.58	97.26±1.08	100.84±1.56	99.38±1.78	0.04	≤7 versus 8-10, *P*=0.028
All fields (superior-hemifield)						
Female	100.91±3.1	101.32±2.95	107.26±1.95	99.21±2.01	0.09	
Male	101.4 (97.2–106.4)	95 (91.6–99.6)	98.9 (93.2–102.4)	98.7 (90–102.7)	0.04	≤7 versus 10-14, *P*=0.002
All fields (inferior-hemifield)						
Female	100.89±3.35	103.78±2.48	112.27±2.4	104.55±1.73	0.01	≤7 versus 10-14, *P*=0.02
Male	104.08±1.57	97.83±0.93	101.86±1.5	100.03±1.65	0.02	≤7 versus 8-10, *P*=0.016

P<0.001 was considered statistically significant (Bonferroni adjustment)

the presence of gonadal hormone receptors in different layers of the retina. Nevertheless, due to the descriptive nature of this study, the mentioned impact of gender on retinal layers should be carefully considered and have to be confirmed by well-designed animal and prospective human studies.

Histologic and optical coherence studies and tomography studies largely agree on foveal development. Formation of the foveal center and deepening of the foveal pit occur at 31- and 42-weeks' postmenstrual age owing to centrifugal inner retinal cell displacement.[22,23] Simultaneously, the inner segment and outer segment bands of the outer retina undergo gradual development or remodeling in the opposite route.[23,24] There was no comparable data for comparison in older children.

The assessment of the mid-retina (inner nuclear layer to OPL, [INL-OPL]) in retinal illness is important, as it has been documented in diabetic patients.[25] The INL is mainly formed by the nuclei of bipolar and Müller cells and by the association of horizontal and amacrine cells.

At the foveal area, MRT thinning is concordant with IRT thickening. We hypothesize that bipolar, amacrine, horizontal, and Müller cells were modestly decreasing in this area up to the age of 14 years while ganglion cell density was increasing. There is a sudden increase in MRT over the age of 14 years,

Table 3: Middle retinal thickness of normal children and adolescents in different age groups presented separately for both sexes per micrometer

	≤7	8–10	11–14	>14	Significant	*Post-hoc* tests
Fovea						
Female	42.1 (39.9–48.9)	38.3 (35.7–45.8)	42.6 (36–49.5)	43.1 (39.4–53.1)	0.45	
Male	42.34±2.06	41.66±0.92	43.8±0.96	43.22±1.3	0.28	
Parafovea (superior-hemifield)						
Female	70.56±3.11	69.75±2.19	71.59±2.8	73.7±2.73	0.81	
Male	69.8 (65.2–73.1)	69.1 (66–73.4)	70.2 (66.3–78.2)	75 (67.2–79.6)	0.49	
Parafovea (inferior-hemifield)						
Female	79.2 (75–82.1)	73.6 (67–85.2)	70.1 (67.7–77)	69 (64.1–87.9)	0.35	
Male	73.4 (70.5–81.6)	69.3 (65.5–75.2)	66.2 (65.4–68.5)	72.8 (65.8–83.6)	<0.001	11–14 versus ≤7, *P*=0.001 11–14 versus >14, *P*=0.03
Perifovea (temporal)						
Female	60.91±1.45	56.22±0.78	58.09±1.11	57.34±1.17	0.08	
Male	58.89±0.69	56.36±0.61	56.37±0.71	57.09±0.76	0.055	
Perifovea (superior)						
Female	64.8 (56.6–65.9)	56.7 (54.7–64)	59.8 (54.5–64)	55.7 (54.1–58.7)	0.12	
Male	58.32±0.85	57.6±0.91	57.35±1.13	58±1.12	0.85	
Perifovea (nasal)						
Female	64.4±2.66	62.84±2.45	62.52±1.04	60.31±2.1	0.63	
Male	63.4 (59–66.1)	58.8 (56.5–64.7)	58.1 (56.1–62.7)	61.8 (56.8–66.2)	0.055	
Perifovea (inferior)						
Female	63.2 (61.1–67.9)	60.5 (56.2–68.6)	59.7 (56.8–62.5)	55.4 (51.6–59.7)	0.02	≤7 versus 11–14, *P*<0.011
Male	62.4 (58.2–66.4)	56.5 (54–61.2)	55.3 (53–57.6)	58.7 (55.2–61)	<0.001	≤7 versus 8–10, *P*=0.03 ≤7 versus 11–14, *P*<0.001
Perifovea (superior-hemifield)						
Female	63.3 (56.5–66.3)	56.8 (54.8–63.6)	59.6 (55.7–64)	57.4 (55.5–59.1)	0.24	
Male	59.15±0.67	58.18±0.78	57.79±0.87	59.05±1.03	0.45	
Perifovea (inferior-hemifield)						
Female	63.64±1.38	62.14±2.91	60.12±0.71	57.66±1.84	0.11	
Male	62.13±0.75	58.71±1.06	56.41±0.78	59.41±1.1	<0.001	≤7 versus 11–14, *P*=0.001
All fields (superior-hemifield)						
Female	60.47±1.96	57.6±1.3	59.49±1.51	57.33±0.95	0.47	
Male	58.28±0.69	57.74±0.79	57.73±0.94	58.62±1.02	0.84	
All fields (inferior-hemifield)						
Female	63.17±1.09	60.59±2.15	59.25±0.71	57.54±1.96	0.1	
Male	61.46±0.84	57.79±0.97	55.71±0.75	58.93±1	<0.001	≤7 versus 8–10, *P*=0.02 ≤7 versus 10–14, *P*=0.001

P<0.001 was considered statistically significant (Bonferroni adjustment)

accompanied by a modest reduction in IRT. Parafoveal and perifoveal alterations were also observed in the fovea. These changes may be caused by changes in mid-retina cells caused by adolescent sex and growth hormones. A new possibility for using growth hormones in retinal regeneration studies has been revealed. Growth hormone acts as a synaptogenic modulator in the chick retina.[26,27]

In the current investigation, the ORT in the central 1 mm area (165.17 ± 10.63) was much less than the levels informed by Yanni *et al*.(210.2 ± 2.4) in children aged 5–16 years using Spectralis SD-OCT (Heidelberg Engineering; Vista, CA).[8] Those might be due to different instruments used or ethnic and racial variances.

Studies have revealed that the development of the fovea centralis is a lengthy process that continues for several years after birth.[22] Histological investigations indicate increasing thickening of the foveal ONL after birth, as cone packing and elongation of IS and OS occur.[24] According to Thomas *et al*., cone-packing density in the fovea only reaches half-adult levels at 45 months.[2] The photoreceptor subcellular structures in the foveal center progress

Table 4: Outer retinal thickness of normal children and adolescents in different age groups presented separately for both sexes per micrometer

	≤7	8-10	11-14	>14	Significant	*Post-hoc* tests
Fovea						
Female	163.13±5.32	164.38±2.56	165.41±3.13	167.6±3.32	0.87	
Male	162.33±2.5	162.76±1.96	166.83±1.89	169.64±2.92	0.15	
Parafovea (superior-hemifield)						
Female	148.2±3.94	145.54±1.65	148.55±1.58	147.28±3.13	0.79	
Male	146.24±2.63	144.2±2.24	144.16±1.88	145.36±2.77	0.92	
Parafovea (inferior-hemifield)						
Female	136.21±6.02	137.25±3.27	144.46±3.26	144.84±4.2	0.3	
Male	138.76±3.11	142.08±1.64	145.67±2.17	141.41±2.94	0.24	
Perifovea (temporal)						
Female	132.39±4.42	130.41±0.7	134.1±1.51	134.39±0.96	0.02	8–10 versus >14, P=0.02
Male	134.53±1.63	132.37±1.25	133.79±1.29	133.42±1.56	0.74	
Perifovea (superior)						
Female	136.66±3.52	133.93±1.11	139.36±1.28	138.63±1.54	0.03	8–10 versus 11–14, P=0.01
Male	135 (129.9–143.6)	135.5 (131.5–141.5)	134.8 (128.9–142.9)	136.2 (130.6–142.5)	0.96	
Perifovea (nasal)						
Female	131.06±3.49	127.35±1.67	136.66±1.57	133.51±2.61	0.02	8–10 versus 11–14, P=0.01
Male	131.92±2.19	132.84±1.32	133.81±1.68	128.6±2.34	0.32	
Perifovea (inferior)						
Female	121.69±4.33	121.95±1.32	129.11±2.09	128.53±2.2	0.03	≤7 versus >14, P≤0.015
Male	124.26±1.97	126.95±1.03	127.95±1.46	124.29±2.03	0.25	
Perifovea (superior-hemifield)						
Female	135.47±3.53	132.5±0.79	138.07±1.27	137.26±1.37	0.01	≤7 versus 11–14, P=0.007 ≤7 versus >14, P=0.048
Male	135.93±1.78	135.05±1.25	135.29±1.31	134±1.64	0.91	
Perifovea (inferior-hemifield)						
Female	125.44±4.09	124.33±1.26	131.54±1.78	130.28±2.08	0.06	
Male	127.7±1.8	129.12±1	130.29±1.43	126.87±1.97	0.41	
All fields (superior-hemifield)						
Female	137.6±3.58	134.31±0.94	139.79±1.38	138.7±1.55	0.14	
Male	137.62±1.92	136.91±1.32	136.49±1.36	135.89±1.68	0.94	
All fields (inferior-hemifield)						
Female	126.97±4.32	127.16±1.51	132.94±2.01	132.63±2.44	0.17	
Male	129.31±1.96	131.46±1.01	132.4±1.46	129.04±2	0.37	

P<0.001 were considered significant (Bonferroni adjustment)

centripetally as the baby grows.[28] Agreeing with Thomas, we can assume that the observed cone packing and probably remodeling, and development may continue until adulthood.[2] The height of photoreceptors rises steadily from childhood to adulthood, resulting in thin and long cones in the foveal center and long rod outer segments with eccentricity.[28] Further OCT investigations on normal foveal development in term newborns and young children revealed that the ONL continues to thicken at least until the age of 12 years, indicating an increasing number of foveal cone photoreceptors.[29]

The main limitation of our study was the relatively small number of cases and its nonlongitudinal nature. Future studies with longer follow-up periods are needed to evaluate the repeatability and reproducibility of segmented macular layer measurements in children. Our findings were also confined to the macular region of the retina, and while the macula is crucial for central vision, it only accounts for a small fraction of the total retinal area. To better understand the normative morphology of peripheral retinal regions in childhood, and expected changes in morphology over time, wide-field scanning methods are necessary. Furthermore, we did not examine axial length, even though the axial length appears to have minimal influence on macular and retinal nerve fiber layer thickness measurements.[9,30]

MT appears to change regularly, indicating that the area is plastic. As a result of this study, a valid normative database of segmented MTs of healthy Iranian children and adolescents can be generated. The RT of children increased as they grew older, and gender also played a role in layered thicknesses. Up to the age of 14 years, the inner retina of the macular area thickened while the MRT shrank, with reciprocal changes at older ages.

Acknowledgments

The authors would like to thank all staff in the imaging center, at Farabi Eye Hospital, Tehran, Iran, for their kind assistance during this study. The author(s) thank any study participant(s) for their involvement in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bringmann A, Syrbe S, Görner K, Kacza J, Francke M, Wiedemann P, *et al.* The primate fovea: Structure, function and development. Prog Retin Eye Res 2018;66:49-84.
2. Thomas MG, Papageorgiou E, Kuht HJ, Gottlob I. Normal and abnormal foveal development. Br J Ophthalmol 2022;106:593-9.
3. Dubis AM, Hansen BR, Cooper RF, Beringer J, Dubra A, Carroll J. Relationship between the foveal avascular zone and foveal pit morphology. Invest Ophthalmol Vis Sci 2012;53:1628-36.
4. Provis JM, van Driel D. Retinal development in humans: The roles of differential growth rates, cell migration and naturally occurring cell death. Aust N Z J Ophthalmol 1985;13:125-33.
5. Wilk MA, Wilk BM, Langlo CS, Cooper RF, Carroll J. Evaluating outer segment length as a surrogate measure of peak foveal cone density. Vision Res 2017;130:57-66.
6. Polaczek-Krupa B, Grabska-Liberek I. Application of retinal thickness analyzer (RTA) in diagnosis and treatment monitoring in retinal diseases. Klin Oczna 2006;108:452-6.
7. Kafieh R, Rabbani H, Hajizadeh F, Abramoff MD, Sonka M. Thickness mapping of eleven retinal layers segmented using the diffusion maps method in normal eyes. J Ophthalmol 2015;2015:259123.
8. Yanni SE, Wang J, Cheng CS, Locke KI, Wen Y, Birch DG, *et al.* Normative reference ranges for the retinal nerve fiber layer, macula, and retinal layer thicknesses in children. Am J Ophthalmol 2013;155:354-60.e1.
9. Turk A, Ceylan OM, Arici C, Keskin S, Erdurman C, Durukan AH, *et al*. Evaluation of the nerve fiber layer and macula in the eyes of healthy children using spectral-domain optical coherence tomography. Am J Ophthalmol 2012;153:552-9.e1.
10. Ghassemi F, Mirshahi R, Bazvand F, Fadakar K, Faghihi H, Sabour S. The quantitative measurements of foveal avascular zone using optical coherence tomography angiography in normal volunteers. J Curr Ophthalmol 2017;29:293-9.
11. Yau GS, Lee JW, Woo TT, Wong RL, Wong IY. Central macular thickness in children with myopia, emmetropia, and hyperopia: An optical coherence tomography study. Biomed Res Int 2015;2015:847694.
12. Pröbster C, Tarau IS, Berlin A, Kleefeldt N, Hillenkamp J, Nentwich MM, *et al.* Quantitative fundus autofluorescence in the developing and maturing healthy eye. Transl Vis Sci Technol 2021;10:15.
13. Bafiq R, Mathew R, Pearce E, Abdel-Hey A, Richardson M, Bailey T, *et al*. Age, sex, and ethnic variations in inner and outer retinal and choroidal thickness on spectral-domain optical coherence tomography. Am J Ophthalmol 2015;160:1034-43.e1.
14. Ooto S, Hangai M, Tomidokoro A, Saito H, Araie M, Otani T, *et al.* Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. Invest Ophthalmol Vis Sci 2011;52:8769-79.
15. El-Dairi MA, Asrani SG, Enyedi LB, Freedman SF. Optical coherence tomography in the eyes of normal children. Arch Ophthalmol 2009;127:50-8.
16. Huynh SC, Wang XY, Rochtchina E, Mitchell P. Distribution of macular thickness by optical coherence tomography: Findings from a population-based study of 6-year-old children. Invest Ophthalmol Vis Sci 2006;47:2351-7.
17. Grover S, Murthy RK, Brar VS, Chalam KV. Comparison of retinal thickness in normal eyes using Stratus and Spectralis optical coherence tomography. Invest Ophthalmol Vis Sci 2010;51:2644-7.
18. Rosenfield RL, Lipton RB, Drum ML. Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. Pediatrics 2009;123:84-8.
19. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol 2016;4:254-64.
20. Na KS, Jee DH, Han K, Park YG, Kim MS, Kim EC. The ocular benefits of estrogen replacement therapy: A population-based study in postmenopausal Korean women. PLoS One 2014;9:e106473.
21. Nuzzi R, Scalabrin S, Becco A, Panzica G. Gonadal hormones and retinal disorders: A review. Front Endocrinol (Lausanne) 2018;9:66.
22. Vajzovic L, Hendrickson AE, O'Connell RV, Clark LA, Tran-Viet D, Possin D, *et al.* Maturation of the human fovea: Correlation of spectral-domain optical coherence tomography findings with histology. Am J Ophthalmol 2012;154:779-89.e2.
23. Maldonado RS, O'Connell RV, Sarin N, Freedman SF, Wallace DK, Cotten CM, *et al.* Dynamics of human foveal development after premature birth. Ophthalmology 2011;118:2315-25.
24. Hendrickson A, Possin D, Vajzovic L, Toth CA. Histologic development of the human fovea from midgestation to maturity. Am J Ophthalmol 2012;154:767-78.e2.
25. Abu El-Asrar AM, Midena E, Al-Shabrawey M, Mohammad G. New developments in the pathophysiology and management of diabetic retinopathy. J Diabetes Res 2013;2013:424258.
26. Martinez-Moreno CG, Epardo D, Balderas-Márquez JE, Fleming T, Carranza M, Luna M, *et al.* Regenerative effect of growth hormone (GH) in the retina after kainic acid excitotoxic damage. Int J Mol Sci 2019;20:4433.
27. Fleming T, Martinez-Moreno CG, Carranza M, Luna M, Harvey S, Arámburo C. Growth hormone promotes synaptogenesis and protects neuroretinal dendrites against kainic acid (KA) induced damage. Gen Comp Endocrinol 2018;265:111-20.
28. Vinekar A, Mangalesh S, Jayadev C, Maldonado RS, Bauer N, Toth CA. Retinal imaging of infants on spectral domain optical coherence tomography. Biomed Res Int 2015;2015:782420.
29. Lee H, Purohit R, Patel A, Papageorgiou E, Sheth V, Maconachie G, *et al. In vivo* foveal development using optical coherence tomography. Invest Ophthalmol Vis Sci 2015;56:4537-45.
30. Tariq YM, Samarawickrama C, Pai A, Burlutsky G, Mitchell P. Impact of ethnicity on the correlation of retinal parameters with axial length. Invest Ophthalmol Vis Sci 2010;51:4977-82.

Supplementary Table 1: Inter-eye Pearson's correlation coefficients for different retinal layers of both eyes

Inter-eye correlation (whole retinal thickness)

Variables	Fovea (OS)	Parafovea (OS)		Perifovea (OS)		All field (OS)	
		Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi
Fovea (OD)							
Pearson Correlation	-0.06	-0.21	-0.17	-0.11	-0.17	-0.11	-0.15
Sig. (2-tailed).	0.64	0.09	0.18	0.38	0.17	0.37	0.25
Parafovea (OD) (Sup-hemi)							
Pearson Correlation	0.02	-0.08	-0.08	0.06	-0.01	0.03	0
Sig. (2-tailed).	0.88	0.51	0.52	0.62	0.96	0.81	0.99
Parafovea (OD) (Inf-hemi)							
Pearson Correlation	0	-0.07	-0.09	0.06	-0.02	0.04	-0.01
Sig. (2-tailed).	0.98	0.59	0.48	0.64	0.88	0.75	0.96
Perifovea (OD) (Sup-hemi)							
Pearson Correlation	-0.02	0.06	0.04	0.25*	0.21	0.23	0.23
Sig. (2-tailed).	0.86	0.64	0.78	0.05	0.09	0.07	0.07
Perifovea (OD) (Inf-hemi)							
Pearson Correlation	1.00	0.06	0.04	0.22	0.17	0.20	0.18
Sig. (2-tailed).	0.84	0.63	0.75	0.08	0.19	0.11	0.15
All field (OD) (Sup-hemi)							
Pearson Correlation	-0.02	0.03	0.01	0.22	0.18	0.19	0.19
Sig. (2-tailed).	0.85	0.80	0.91	0.09	0.17	0.13	0.14
All field (OD) (Inf-hemi)							
Pearson Correlation	0.00	0.02	0.00	0.16	0.11	0.15	0.13
Sig. (2-tailed).	0.98	0.89	0.98	0.20	0.39	0.23	0.31

Inter-eye correlation (inner retinal thickness)

Variables	Fovea (OS)	Parafovea (OS)		Perifovea (OS)		All field (OS)	
		Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi
Fovea (OD)							
Pearson Correlation	0.05	-0.15	-0.14	-0.16	-0.24	-0.17	-0.2
Sig. (2-tailed)	0.72	0.25	0.28	0.19	0.06	0.18	0.11
Parafovea (OD) (Sup-hemi)							
Pearson Correlation	0.14	-0.02	-0.05	0.03	0.01	-0.01	0.00
Sig. (2-tailed)	0.28	0.85	0.68	0.8	0.95	0.93	0.98
Parafovea (OD) (Inf-hemi)							
Pearson Correlation	0.10	0.02	0.00	0.03	0.01	0.01	0.02
Sig. (2-tailed)	0.44	0.87	0.97	0.84	0.92	0.96	0.9
Perifovea (OD) (Sup-hemi)							
Pearson Correlation	-0.03	0.12	0.08	0.23	0.25	0.22	0.25
Sig. (2-tailed)	0.80	0.34	0.53	0.07	0.05	0.08	0.05
Perifovea (OD) (Inf-hemi)							
Pearson Correlation	0.07	0.18	0.17	0.22	0.26*	0.22	0.26*
Sig. (2-tailed)	0.57	0.16	0.18	0.08	0.03	0.08	0.03
All field (OD) (Sup-hemi)							
Pearson Correlation	-0.01	0.11	0.07	0.20	0.22	0.18	0.22
Sig. (2-tailed)	0.94	0.39	0.59	0.11	0.08	0.14	0.09
All field (OD) (Inf-hemi)							
Pearson Correlation	0.06	0.14	0.13	0.17	0.20	0.16	0.20
Sig. (2-tailed)	0.62	0.26	0.30	0.19	0.11	0.20	0.11

Inter-eye correlation (middle retinal thickness)

Variables	Fovea (OS)	Parafovea (OS)		Perifovea (OS)		All field (OS)	
		Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi
Fovea (OD)							
Pearson Correlation	0.03	-0.16	-0.01	-0.18	-0.03	-0.20	-0.04

Contd...

Supplementary Table 1: Contd...

Inter-eye correlation (middle retinal thickness)

Variables	Fovea (OS)	Parafovea (OS)		Perifovea (OS)		All field (OS)	
		Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi
Sig. (2-tailed)	0.83	0.21	0.95	0.15	0.79	0.10	0.75
Parafovea (OD) (Sup-hemi)							
Pearson Correlation	0.05	0.04	0.18	0.09	0.15	0.06	0.16
Sig. (2-tailed)	0.70	0.77	0.15	0.50	0.23	0.62	0.20
Parafovea (OD) (Inf-hemi)							
Pearson Correlation	-0.10	-0.11	0.00	-0.08	0.03	-0.06	0.05
Sig. (2-tailed)	0.44	0.38	0.99	0.55	0.82	0.65	0.67
Perifovea (OD) (Sup-hemi)							
Pearson Correlation	0.03	0.06	0.19	0.13	0.14	0.11	0.17
Sig. (2-tailed)	0.79	0.65	0.13	0.29	0.26	0.37	0.19
Perifovea (OD) (Inf-hemi)							
Pearson Correlation	-0.07	-0.12	0.12	-0.07	0.05	-0.07	0.1
Sig. (2-tailed)	0.60	0.36	0.35	0.59	0.72	0.57	0.42
All field (OD) (Sup-hemi)							
Pearson Correlation	0.05	0.07	0.19	0.14	0.16	0.12	0.17
Sig. (2-tailed)	0.69	0.60	0.14	0.27	0.22	0.35	0.18
All field (OD) (Inf-hemi)							
Pearson Correlation	-0.07	-0.11	0.08	-0.08	0.02	-0.07	0.08
Sig. (2-tailed)	0.57	0.38	0.54	0.55	0.86	0.58	0.54

Inter-eye correlation (outer retinal thickness)

Variables	Fovea (OS)	Parafovea		Perifovea		All field	
		Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi	Sup-hemi	Inf-hemi
Fovea (OD)							
Pearson Correlation	0.01	-0.07	0.10	-0.06	0.05	-0.09	0.07
Sig. (2-tailed)	0.93	0.58	0.44	0.63	0.70	0.48	0.59
Parafovea (OD) (Sup-hemi)							
Pearson Correlation	0.15	0.12	0.23	0.13	0.22	0.10	0.24
Sig. (2-tailed)	0.23	0.35	0.06	0.30	0.08	0.41	0.06
Parafovea (OD) (Inf-hemi)							
Pearson Correlation	-0.09	-0.12	0.00	-0.07	-0.03	-0.09	-0.03
Sig. (2-tailed)	0.49	0.35	0.97	0.60	0.80	0.46	0.84
Perifovea (OD) (Sup-hemi)							
Pearson Correlation	0.17	0.12	0.24	0.18	0.24	0.15	0.25*
Sig. (2-tailed)	0.18	0.34	0.05	0.15	0.06	0.23	0.05
Perifovea (OD) (Inf-hemi)							
Pearson Correlation	0.02	-0.01	0.13	0.08	0.12	0.05	0.13
Sig. (2-tailed)	0.87	0.96	0.29	0.52	0.37	0.67	0.31
All field (OD) (Sup-hemi)							
Pearson Correlation	0.15	0.10	0.22	0.14	0.22	0.12	0.23
Sig. (2-tailed)	0.22	0.42	0.08	0.26	0.08	0.36	0.07
All field (OD) (Inf-hemi)							
Pearson Correlation	-0.02	-0.02	0.05	0.05	0.06	0.03	0.07
Sig. (2-tailed)	0.88	0.86	0.67	0.67	0.62	0.84	0.60